Autism Spectrum Disorder in Males with Sex Chromosome Aneuploidy: XXY/Klinefelter Syndrome, XYY, and XXYY

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ABSTRACT: ***Objective:*** **Neurodevelopmental concerns in males with sex chromosome aneuploidy (SCA) (XXY/Klinefelter syndrome, XYY, XXYY) include symptoms seen in autism spectrum disorder (ASD), such as language impairments and social difficulties. We aimed to: (1) evaluate ASD characteristics in research cohorts of SCA males under DSM-IV compared to DSM-5 criteria, and (2) analyze factors associated with ASD diagnoses in SCA.** ***Methods:*** **Evaluation of participants with XXY/KS (n=20), XYY (n=57) and XXYY (n=21) included medical history, cognitive/adaptive testing, Social Communication Questionnaire, Social Responsiveness Scale, Autism Diagnostic Observation Schedule, Autism Diagnostic Interview-Revised, and DSM ASD criteria. Clinical impressions of ASD diagnostic category using the ADOS and DSM-IV criteria were compared to ADOS-2 and DSM-5 criteria. T-tests compared cognitive, adaptive, SES and prenatal vs. postnatal diagnoses between ASD and no ASD groups.** ***Results:*** **ASD rates in these research cohorts were 10% in XXY/KS, 38% in XYY, and 52% in XXYY using ADOS-2/DSM-5, and were not statistically different compared to DSM-IV criteria. In XYY and XXYY, the ASD group had lower verbal IQ and adaptive functioning compared to those without ASD. Many children without ASD still showed some social difficulties.** ***Conclusion:*** **ASD rates in males with SCA are higher than reported for the general population. Males with Y chromosome aneuploidy (XYY and XXYY) were 4.8 times more likely to have a diagnosis of ASD than the XXY/KS group, and 20 times more likely than males in the general population (1 in 42 males, CDC 2010). ASD should be considered when evaluating social difficulties in SCA. Studies of SCA and Y-chromosome genes may provide insight into male predominance in idiopathic ASD.**

*(J Dev Behav Pediatr 38:*197–207, 2017) **Index terms:** XXY, Klinefelter syndrome, XYY, XXYY, autism spectrum disorder, Y chromosome.

Autism spectrum disorder (ASD) is a behaviorally defined neurodevelopmental disorder characterized by varying degrees of deficits in domains of social communication, reciprocal social interaction, and restricted, repetitive patterns of behavior. With the publication of the DSM-5 in May 2013, there was a significant change in the diagnostic criteria of ASD to reflect the commonalities and interrelationships of these core symptoms across a wide spectrum of severities, and all cases are now classified under a single diagnosis of ASD rather than the distinct disorders in the DSM-IV of Autistic Disorder, Asperger Syndrome, and Pervasive Developmental Disorder—Not Otherwise Specified (PDD-NOS). Furthermore, the DSM-5 diagnostic criteria also emphasize the importance of neurobiological and genetic factors that may underlie the ASD symptoms, and the presence of any genetic disorders or other medical conditions known to be associated with ASD are now part of the "specifiers" to be included in the diagnostic description. This has led to an increased interest in genetic disorders with behavioral phenotypes that include increased rates of ASD and other social and/or communication impairments.

Genetic studies in children with ASD identify an etiologic diagnosis in approximately 10 to up to 40% of cases, for example, chromosomal duplications and deletions, fragile X syndrome, and mutations in specific genes such as MECP2 or PTEN mutations.[1] In many studies evaluating genetic etiologies in cohorts of children with ASD, cases of sex chromosome aneuploidy (SCA) conditions including XXY/Klinefelter Syndrome (XXY/KS), XYY, and XXYY have been identified.[2–4] There have also been many case reports and case series of ASD in males with XXY/KS, XYY, and XXYY.[5–8]

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XXY/KS, XYY, and XXYY are the 3 most common forms of SCA in males. XXY/KS occurs in approximately 1/650 male births,[9,10] and is characterized by medical features of tall stature, hypogonadism (microorchidism and testosterone deficiency), and decreased fertility. Longitudinal studies of birth cohorts have characterized the large spectrum of the psychological and behavioral phenotype in males with XXY/KS. Early speech and language delays are present in up to 70% of children with XXY/KS, and cognitive abilities are typically in the average range, but often show language-based learning disabilities and a lower verbal IQ.[11–13] Behavioral characteristics including shyness, social immaturity, anxiety, and other social difficulties are commonly described.[14]

More recent studies specifically evaluating social functioning in XXY/KS have identified an increased rate of deficits that overlap with those seen in ASD. For example, Van Rijn et al.[15] have described deficits in social cognitive functioning and increased autism traits in adult men with XXY/KS ascertained through support groups. Bishop et al.[16] reported a previous clinical diagnosis of ASD in 11% of 19 boys with prenatal diagnoses of XXY/KS, and, in 2012, a similar rate of 12% positive scores on an autism screening measure (Social Communication Questionnaire; SCQ) in a cohort of 34 pediatric patients with XXY/KS.[17] A similar rate of 11.6% has since been reported by Samango-Sprouse et al.[18] in a separate cohort of boys with a prenatal diagnosis of XXY/KS using a different screening measure (Gilliam Autism Rating Scale score of "very likely"). In 2009, Bruining et al. reported on a study of 51 males age 6 to 20 years with XXY/KS in the Netherlands who were evaluated using the Autism Diagnostic Interview–Revised (ADI-R), and in this cohort, 27% were identified as possibly having ASD. To our knowledge, the Bruining et al.[19] study has been the only study to move beyond ASD screening questionnaires and directly evaluate ASD characteristics in XXY/KS using a gold-standard instrument.

XYY syndrome occurs in approximately 1/1000 male births, and shares features of tall stature, early speech and motor delays, and a high prevalence of language-based learning disabilities with XXY/KS.[20] However, males with XYY do not typically have hypogonadism or fertility problems that occur in XXY/KS. Prospective studies on birth cohorts identified average cognitive abilities, and behavioral traits including hyperactivity and low frustration tolerance. Poor social relatedness, attention deficit hyperactivity disorder, PDD-NOS, and autistic behaviors have been reported in other behavioral studies and case reports in XYY syndrome, with some cohorts reporting rates of previous clinical diagnoses of ASD ranging from 20% to 30%.[21,22] Administration of the SCQ screening questionnaire to a cohort of 22 pediatric patients with XYY led to positive results in 50%.[17] However, no study including direct diagnostic evaluation of children with XYY using standardized autism assessment tools has previously been reported.

XXYY syndrome occurs in approximately 1/18,000 male births. Originally described as a variant of Klinefelter syndrome (XXY) because of shared features of tall stature and hypogonadism,[22] it is now recognized that XXYY is associated with more significant cognitive deficits and behavioral disorders, as well as a higher rate of associated medical problems such as seizures or congenital defects.[23] Due to the low prevalence of XXYY syndrome, there are not prospective studies of birth cohorts like in XXY/KS and XYY. Overall cognitive abilities in XXYY syndrome are typically in the borderline range; however, verbal cognition and language abilities are usually significantly lower than visual-spatial abilities. Previous studies have described behavioral characteristics including impulsivity, difficulty with social relationships, anxiety, and autistic behaviors.[24–26] In a large review of 95 individuals with XXYY by our group, 23% had been previously diagnosed with ASD on presentation to the study.[23]

Taken together, previous studies have shown that the behavioral phenotype of males with SCA includes many features that overlap with those also associated with ASD, including speech/language disorders, verbal cognitive deficits, social deficits, and other autistic behaviors. Moreover, rates of ASD characteristics in SCA are higher than expected compared with the general population when screening measures for ASD are used. However, with the exception of 1 study in XXY/KS, previous studies have not directly evaluated and compared children with SCA using standardized ASD diagnostic measures. The screening measures used in previous studies were developed for use in consideration of the DSM-IV diagnostic criteria, and diagnostic criteria for ASD changed with the publication of the DSM-5 in 2013.[27] Furthermore, the most commonly used standardized assessment tool for ASD called the Autism Diagnostic Observation Schedule (ADOS) was updated and released with revised scoring algorithms as the ADOS-2 in 2012 in anticipation of the changes in the DSM-5 criteria.[28] These changes in both the diagnostic criteria and assessment measures raise the question of how the new criteria have affected diagnostic rates of ASD in males with SCA.

In this project, we aimed to describe 2 studies that directly evaluated diagnostic rates and characteristics of ASD in research cohorts of males with SCA using standardized assessments under the DSM-IV criteria. We aimed to compare diagnostic rates in the same cohorts using updated DSM-5 diagnostic criteria and ADOS-2 assessment algorithms. We hypothesized that rates of ASD would be (1) higher in males with SCA compared with the general population, (2) similar in the 2 trisomy conditions (XXY/KS and XYY), but (3) higher in XXYY compared with the trisomies given more severe neurodevelopmental involvement. Furthermore, we hypothesized that more males with SCA would meet DSM-5 criteria for ASD compared with DSM-IV. We also aimed to analyze factors that could be associated with DSM-5 ASD diagnosis (including SCA subgroup, timing of SCA

diagnosis (prenatal vs postnatal), cognitive abilities, adaptive functioning, and SES).

METHODS

Study Sites

The described studies were independently initiated and conducted at University of California (UC) Davis MIND Institute in Sacramento, CA, and Thomas Jefferson University (TJU) in Philadelphia, PA, with ongoing analysis of the UC Davis cohort subsequently conducted at University of Colorado because of change in institution of the investigator (NT). On discovery of similar research efforts and protocols during data collection, investigators collaborated to combine results from both studies to increase sample size. Minor differences in study protocols between sites are described below and in Figure 1.

Subjects

IRB approval was obtained at all institutions, parents or primary guardians signed consent forms, and participants signed assent forms as appropriate to age and developmental level. In both studies, participants were recruited from hospital-based outpatient clinics and from national sex chromosome aneuploidy (SCA) support organizations for participation. To minimize recruitment bias toward more affected individuals or those with concerns related to autism, recruitment materials indicated that the studies were evaluating features of health and development in SCA, and did not include specific wording that autism-related disorders were being evaluated. Inclusion required confirmation of genetic testing results showing a karyotype of nonmosaic XXY/KS, XYY, or XXYY. In the UC Davis/Colorado study, 63 male children and young adults between the ages of 3 and 25 with XXY/KS (n = 20), XYY (n = 22), or XXYY (n = 21) participated. One male with XYY was found to also have another genetic disorder (distal 22q11.2 deletion syndrome) and was excluded from the study,[29] leaving a total of 62 participants. At TJU, 41 males with XYY participated. Five participants enrolled in both studies, and data from the UC Davis/Colorado study were used in analysis, leaving a total of 36 participants in the TJU study.

Research Assessments

After the participants and/or their parents provided informed consent, participants were evaluated through protocols consisting of 2 main components: (1) psychological assessment, which included cognitive testing and autism assessments by research-reliable evaluators blinded to SCA group and (2) medical history, developmental-behavioral history, and physical examination for all participants.

Psychological Assessments

UC Davis/Colorado Study

In the UC Davis/Colorado study, cognitive levels were estimated using the Mullen Scales of Early Learning (MSEL) for children 3 to 5 years of age, and the Wechsler Abbreviated Scale of Intelligence (WASI) for children aged 6 and above. The MSEL is a standardized developmental assessment that includes subscales of development (visual reception, fine motor, expressive language, receptive language), and allows for a summary score to be calculated.[30] The WASI is an abbreviated cognitive assessment that includes 4 subscales, and provides estimates of verbal IQ (VIQ), performance IQ (PIQ), and full scale IQ (FSIQ).[31] Results of the WASI VIQ, PIQ, and FSIQ have been shown to have good correlation with the more extended Wechsler Intelligence Scale for Children—Fourth Edition (WISC-IV) (r = .85, .78, and .86, respectively).[31]

Adaptive functioning was assessed using the Vineland Adaptive Behavior Scales, second edition, interview

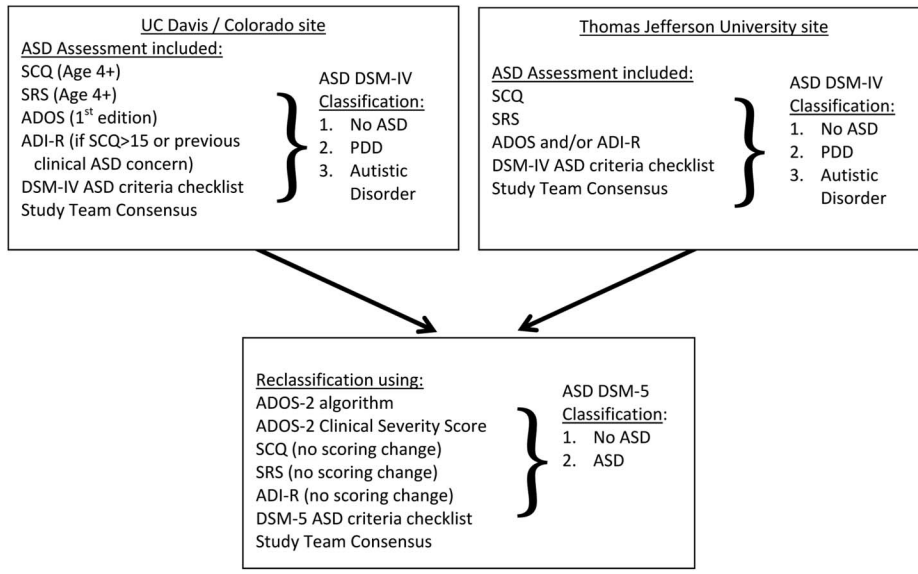

Figure 1. Components of autism spectrum disorder (ASD) assessment and reclassification from DSM-IV to DSM-5 criteria at each site.

edition. The scale is norm referenced, standardized on a national sample, and provides standardized scores of adaptive functioning in domains of communication, daily living skills, and social skills.[32] Socioeconomic status (SES) was assessed using the Hollingshead 2-factor model of parental education and occupation.[33]

Autism Spectrum Disorder–Specific Assessments

Assessment of autism spectrum disorder (ASD) under the DSM-IV criteria was conducted by an interdisciplinary team including Developmental-Behavioral Pediatrics, Child Psychology and a Licensed Clinical Social Worker. All participants were evaluated using the Social Communication Questionnaire (SCQ), the Social Responsiveness Scale (SRS), and the Autism Diagnostic Observation Schedule (ADOS). The SCQ is a 40-item parent-report questionnaire designed to screen for the diagnostic features of ASD based on the ADI-R in children aged 4 and above.[34] A score of 15 or greater has a high sensitivity and specificity for ASD, and indicates the need for further ASD assessment. The SRS is a parent-report questionnaire for children aged 4 and above that evaluates social behaviors across multiple domains as they occur in natural social settings to identify the presence and degree of social deficits and determine whether symptoms are consistent with those seen in ASD.[35] The SRS yields 5 subscale T-scores in the domains of social awareness, social cognition, social communication, social motivation, and autistic mannerisms, and an SRS total score. If screening on the SCQ was positive (score of 15 or greater), if the ADOS results were in the ASD range, or if a previous concern for an ASD was raised by an educational, medical, or psychological professional, the Autism Diagnostic Interview-Revised (ADI-R) was also administered.

The ADOS is a semi-structured interaction session using developmentally appropriate social and play-based interaction and standard activities in a 40- to 60-minute session designed to allow for the observation of behaviors relevant to the diagnosis of ASD. Different modules are selected based on participant age and language level, and all 4 modules were used in this study. The ADOS was validated across a wide range of ages and severity levels in autism with psychometric properties showing good interrater reliability.[36] The original ADOS scoring algorithms were used at the time of autism assessments for all participants in the UC Davis study. In the ADOS algorithms, classification of autistic disorder (AUT) or ASD required meeting a cutoff score in the 2 domains of communication and reciprocal social interaction.

The ADOS-2 was released in 2012 in anticipation of changes to ASD diagnostic criteria with publication of the DSM-5.[28] The ADOS-2 uses the same semi-structured, play-based interaction protocol as the original ADOS; however, there are revised scoring algorithms which produce an overall score that consisted of the Social Affect domain (a combination of communication and reciprocal social interaction items) and the Restricted, Repetitive Behavior score (which was not included in the original ADOS scoring algorithms).[37,38] The ADOS-2 also introduced a new clinical severity score (CSS) which allows for the severity of ASD symptoms to be classified and compared between individuals and tracked over time.[37,39] The CSS scoring system takes into account age and language-level (ADOS-2 module administered), and scores are then classified into severity levels as No ASD (0–3), Non-Autism ASD (4–5), and Autism (6–10). All ADOS assessments were rescored using the ADOS-2 algorithms to determine if classification of ASD changed using the revised algorithms. Furthermore, a CSS was calculated for each child as per the ADOS-2 scoring to determine and compare severity of ASD symptoms (Fig. 2).

The ADI-R is a semi-structured, standardized parent interview developed to assess the presence and severity of autism behaviors throughout childhood.[40] Classification of autism requires meeting a cutoff score in 3 domains of Qualitative Abnormalities in Reciprocal Social Interaction, Qualitative Abnormalities in Communication, and Restricted, Repetitive, and Stereotyped Patterns of Behavior. The ADI-R has been carefully validated across a wide range of ages and severity levels. Owing to the time requirement of the ADI-R, only participants with a positive SCQ screen, ADOS scores in the ASD range, or individuals with a previous concern or diagnosis of ASD completed the ADI-R in the UC Davis study.

TJU Study

In the TJU study, participants were 4 to 14 years of age, and cognitive assessment was conducted using the Differential Abilities Scale—Second Edition (DAS-2).[41] The DAS-2 yields an overall score (General Conceptual Ability; [GCA]), and verbal, nonverbal, and spatial composite scores.

The composite scores on the DAS-2 are strongly correlated (r = .71 to .84) to the corresponding WISC-IV domains, i.e., verbal and VIQ scores, nonverbal and PIQ, and GCA and FSIQ scores. SES was measured using the same Hollingshead 2-factor model.[33] Autism assessment at TJU included neurodevelopmental history, SCQ, and SRS for all participants. Standardized autism evaluations at TJU included the ADI-R and/or ADOS (ADI-R + ADOS n = 15, ADI-R only n = 12, and ADOS only n = 9).

At both sites, the study teams first evaluated results of all measures in the assessment battery and records available for each participant to determine assignment by team consensus into 1 of 3 groups: no ASD, Pervasive Developmental Disorder—Not Otherwise Specified, or AUT. No individuals met criteria for Asperger Syndrome because early language delays were present in all children who met criteria for ASD. Protocols were then reviewed and rescored by study teams using both the ADOS-2 algorithms and the revised DSM-5 criteria, and then reclassified into one of 2 diagnostic groups: ASD and no ASD. See Figure 1 for a diagram of the components of ASD assessment battery and reclassification at each site. Table 2 includes results of these analyses. The

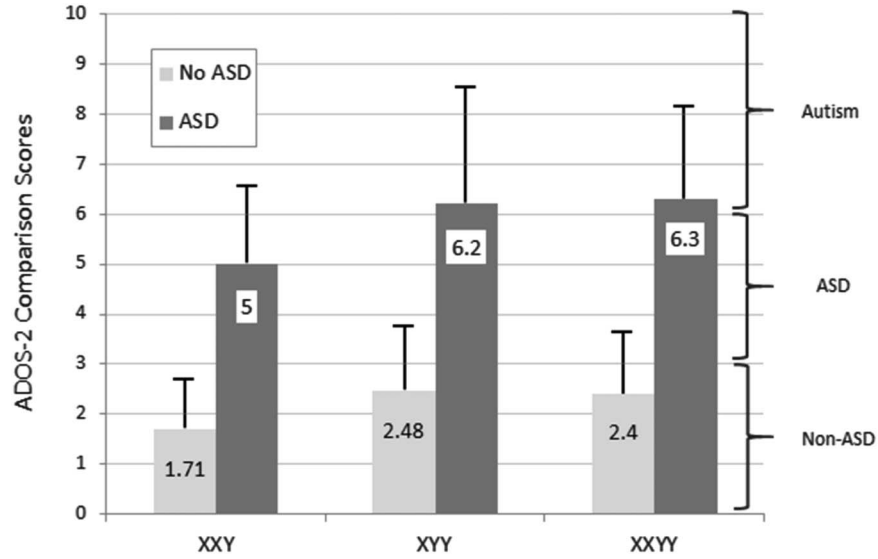

Figure 2. ADOS-2 severity comparison scores for XXY (No autism spectrum disorder [ASD] n = 18, ASD n = 2), XYY (No ASD n = 35; ASD n = 22), XXYY (No ASD n = 10; ASD n = 11). Mean scores in the no ASD group are in the non-ASD range as expected, however, still slightly elevated especially for XYY and XXYY.

CSS was also calculated for all participants using the ADOS-2 scoring system.

Medical/Developmental History and Physical Examination

In both sites, a medical and developmental-behavioral history and physical examination were performed on each subject to characterize developmental milestones, neurodevelopmental history and to identify other factors that may influence neurodevelopment such as prematurity or other chronic medical conditions. Genetic testing results were reviewed, and age at SCA diagnosis was recorded to evaluate differences between those ascertained in the prenatal versus postnatal period. Family history of ASD and other neurodevelopmental disorders were recorded.

Data Analysis

Before testing hypotheses, univariate and bivariate range and logic checks were performed on all variables, using both numerical and graphical summaries. Descriptive statistics were calculated for each SCA group at each site for age, IQ, SES, and psychological assessment results. In the UC Davis study, one-way analysis of variance was conducted to assess significant differences between the 3 SCA groups in age, IQ and adaptive domains, and SES. For those with significance of $p < .05$, post-hoc Tukey or Games-Howell analyses were conducted to determine which pairwise differences were statistically significant (Table 1). Results in the XYY groups at the 2 study locations were compared by independent t tests (age, IQ subscales, SES, SCQ), and Fisher's Exact Test (ASD rates; prenatal vs postnatal diagnosis) (Table 1). The rates of ASD in the 3 SCA groups were compared by χ^2 with a significant p value equal or less than .05 (Table 2). Data from the XYY groups from the 2 studies were pooled, and independent t tests were used to compare age, SES, cognitive subscales, adaptive skills, and timing of ascertainment in the XYY and XXYY groups with ASD and without ASD as determined by DSM-5 criteria (Table 3).

RESULTS

Subjects

A total of 62 participants were included in data analysis (20 XXY/KS, 21 XYY, and 21 XXYY) in the UC Davis/Colorado study, and 36 participants with XYY from the TJU study. Demographics, cognitive subscales, and timing of ascertainment (prenatal vs postnatal) are reported in Table 1. There were no significant differences between the XYY groups from the 2 study sites by age, socioeconomic status (SES), and timing of diagnosis.

In the UC Davis/Colorado study, there were no overall significant differences in age between the 3 groups (F [2,59]= 2.89, $p = .064$). The rates of prenatal versus postnatal diagnosis were not significantly different between XXY/KS and XYY; all subjects in the XXYY group were diagnosed after birth. There were no significant differences in SES or ethnicity between the 3 sex chromosome aneuploidy (SCA) groups. Most participants were white.

Results of cognitive assessment using the WASI in participants aged 6 or above from the UC Davis/Colorado study showed a mean full scale IQ (FSIQ) of 104.7 (SD 10.6) in the XXY/KS group, which was higher than the mean FSIQ in the XYY group of 96.4 (SD 15.4), although this difference did not reach significance ($p = .16$). The verbal IQ was significantly lower in the XYY group compared with XXY/KS ($p = .03$), whereas the performance IQ (PIQ) was not ($p = .76$). As expected, because of added gene dosage effects of the tetrasomy

Table 1. Description of Participants–Demographics and Psychological Assessments

	XXY/KS	XYY[a]	XXYY	Statistical Comparison
UC Davis/Colorado				
N	20	21	21	
Mean age (SD)	9.11 (4.13)	9.44 (4.60)	12.47 (5.77)	F (2,59) = 2.89, p = .064
Age range	3–17	3–21	3–22	
Mean SES	53.4	50.8 (9.2)	51.6	F (2,59) = 0.20, p = .812
Race (% white)	95	90.5	95.2	
SCA diagnosis				
Prenatal	12	8	0	XXY/KS vs XYY: $\chi^2(1) = 1.19$, p = .28[b]
Postnatal	8	13	21	
IQ (WASI), n	15	16	17	
Verbal, mean (SD)	102.7 (11.3)	90.3 (15.2)	79.3 (11.8)	F (2,45) = 14.62, $p < .01$[c]
Verbal range	76–118	56–113	64–98	
Performance, mean (SD)	105.9 (11.0)	102.2 (17.4)	91.7 (12.4)	F (2,45) = 4.57, p = .015[d]
Performance range	89–129	73–136	65–108	
Full scale, mean (SD)	104.7 (10.6)	96.4 (15.4)	83.2 (10.8)	F (2,45) = 12.43, $p < .01$[d]
Full scale range	81–125	66–127	61–98	
Early Learning Composite (MSEL), n	5	5	4	
ELC, mean (SD)	100.4 (5.7)	85.2 (15.0)	77.0 (24.5)	F (2,11) = 2.50, p = .127
ELC range	91–106	68–103	49–103	
Adaptive (Vineland), n	20	21	21	
ABC, mean (SD)	89.7 (10.4)	83.6 (16.4)	74.4 (13.9)	F (2,59) = 6.37, $p < .01$[e]
ABC range	67–107	59–119	50–101	
Thomas Jefferson University				
N	—	36	—	
Mean age (SD)	—	9.6 (3.9)		
Age range		4–14.9	—	
Mean SES (SD)	—	50.4 (10.2)	—	
Race (% white)	—	80	—	
SCA diagnosis				
Prenatal	—	14	—	
Postnatal	—	22	—	
IQ (DAS-2), n	—	36	—	
Verbal, mean (SD)	—	87.2 (15.3)	—	
Verbal range		53–115		
Nonverbal, mean (SD)	—	93.5 (17.7)	—	
Nonverbal range		56–134		
GCA, mean (SD)	—	89.2 (14.8)	—	
GCA range		58–124		

[a]There were no significant differences between sites in age (p = .82), SES (p = .94), VIQ (p = .55), PIQ (p = .13), FSIQ/GCA (p = .16), or proportion with prenatal diagnosis (p = .99). [b]Z-test of proportions (Bonferroni control for multiple comparisons used, significant relationships are $p < .0167$): XXY/KS versus XYY (z = 1.44, p = 1.51), XXY/KS versus XXYY (z = 5.48, p = .000), XYY versus XXYY (z = 3.60, p = .000). [c]Post-hoc Tukey XXY/KS > XYY > XXYY. [d]Post-hoc Tukey XXY/KS, XYY > XXYY. [e]Post-hoc Games-Howell XXY/KS versus XYY ns (p = .35), XXY/KS > XXYY ($p < .01$), XYY versus XXYY ns (p = .13). GCA, General Conceptual Ability; SCA, sex chromosome aneuploidy; SES, socioeconomic status.

condition of XXYY, cognitive and adaptive scores in the XXYY group were significantly lower compared with both XXY/KS and XYY (for FSIQ and VIQ, $p < .001$; for PIQ, p = .01). In all 3 groups, the mean adaptive score was lower than the measured cognitive scores (XXY/KS, 16.6 points; XYY, 15.2 points; XXYY, 8.2 points).

Cognitive assessment in the TJU study was conducted using the DAS-2, a measure that has strong concurrent validity with Wechsler scales (r = .71 to .84). The Mean General Conceptual Abilities score in the XYY group was 89.2 (SD 14.8). There were no significant differences in verbal, nonverbal, or

Table 2. Comparison of ASD Rates in Study Participants with XXY/KS, XYY, and XXYY

	XXY/KS		XYY		XXYY	
	DSM-IV	DSM-5	DSM-IV	DSM-5	DSM-IV	DSM-5
UC Davis/Colorado						
No ASD	19/20 (95%)	18/20 (90%)	14/21 (67%)	12/21 (57%)	12/21 (57%)	10/21 (48%)
ASD	PDD 1/20 (5%)	ASD 2/20 (10%)	PDD 6/21 (29%)	ASD 9/21 (43%)	PDD 6/21 (14%)	ASD 11/21 (52%)
	AUT 0/20 (0%)		AUT 1/21 (4.7%)		AUT 3/21 (25%)	
	TOTAL 1/20 (5%)		TOTAL 7/21 (33%)		TOTAL 9/21 (43%)	
TJU						
No ASD	NA		24/36 (67%)	23/36 (64%)	NA	
ASD	NA		PDD 5/36 (14%)	ASD 13/36 (36%)	NA	
			AUT 7/36 (19%)			
			TOTAL 12/36 (33%)			
Combined sites total ASD[a]	1/20 (5%)	2/20 (10%)	19/57 (33%)	22/57 (38%)	9/21 (43%)	11/21 (52%)

[a]For Combined Sites Total ASD Rate (DSM-5): χ^2 8.79, $p = .012$, post-hoc results showed XYY and XXYY > XXY/KS. ASD, autism spectrum disorder; AUT, autistic disorder; NA, not applicable; PDD, Pervasive Developmental Disorder—Not Otherwise Specified.

full scale cognitive scores in the XYY groups between sites.

Autism Assessment

Results of the autism evaluation in the 3 SCA subgroups from both study locations are shown in Table 2, which compares the diagnostic classification results of all study participants when using the original assessment battery (DSM-IV/ADOS) compared with the reclassification using DSM-5/ADOS-2 criteria applied to each case (Fig. 1). In the UC Davis/Colorado study, there was a significant difference in the overall rate of autism spectrum disorder (ASD) between the 3 SCA conditions using both the DSM-IV (χ^2 7.93, $p = .016$) and DSM-5 comparisons (χ^2 8.79, $p = .012$), with post-hoc analysis showing the difference due to XYY and XXYY > XXY/KS. Using the original DSM-IV results, only 1 participant (5%) of the XXY/KS group met criteria for ASD, compared with 33% (7/21) in the XYY group and 43% (9/21) in the XXYY group. After reclassification using the ADOS-2 and DSM-5 criteria, these rates increased slightly to 10% for XXY/KS, 43% for XYY, and 52% for XXYY. The TJU study showed the same results in their separate XYY cohort, with 33% (12/36) meeting DSM-IV criteria and 36% (13/36) meeting DSM-5 criteria for ASD. When combining data from both studies, males with Y chromosome aneuploidy (XYY and XXYY) were 4.8 times more likely to have a diagnosis of ASD than the group with an extra X (XXY/KS), and 20 times more likely to have a diagnosis of ASD than males in the general population, based on the 2014 CDC studies estimating a rate of ASD in 1 in 42 males (CDC, 2014).

When we specifically compared the ADOS with the ADOS-2 scoring results in the 86 participants who received this measure, 75 (87.2%) maintained their original result of no ASD, ASD, or autism, and 78 (90.7%) maintained their broader classification of either ASD or no ASD. There were 11 participants whose ADOS classification changed between the ADOS and ADOS-2 algorithms. Seven participants changed from a diagnosis of no ASD to ASD because of inclusion of the stereotyped behaviors/restricted interests domain (1 XXY/KS, 4 XYY, 2 XXYY), 3 remained on the autism spectrum however increased from a severity of ASD to autistic disorder (AUT) because of inclusion of the stereotyped behaviors/restricted interests domain (2 XYY, 1 XXYY), and 1 patient with XYY changed from an ADOS classification of ASD to No ASD on the ADOS-2 because of elimination of items A6 (using of another person's body as a tool) and B4 (integration of eye contact with words/vocalizations during social interactions) scores from the Module 1 algorithm. Overall, these results suggest that the ADOS-2 may classify slightly more children with SCA in the ASD range compared with the original ADOS algorithm (mainly because of the inclusion of stereotyped behaviors/restricted interests in the total score); however, the differences in positive ASD rates comparing the 2 versions did not reach statistical significance.

Table 3. Comparison of ASD Versus No ASD in XYY and XXYY Based on DSM-5 Results

	XYY			XXYY		
Mean	**ASD (n = 22)**	**No ASD (n = 35)**	***p***	**ASD (n = 11)**	**No ASD (n = 10)**	***p***
Age	9.16	9.88	.50	13.7	11.5	.38
SES	52.1	49.4	.35	52.3	50.1	.42
Verbal IQ	84.6	93.7	.03*	68.9	83.3	.03*
Performance IQ	91.0	99.5	.10	86.5	94.6	.21
Full scale IQ	87.2	94.0	.11	76.0	86.3	.09
Adaptive functioning	71.0	91.0	<.01*	65.6	81.0	<.01*
Ascertainment	Pre: 7/22; Post: 15/22	Pre: 15/35 Post: 20/35	.44	Post: 11/11	Post: 10/10	

*Significance level $p < .05$. ASD, autism spectrum disorder; Pre, prenatal; Post, Postnatal; SES, socioeconomic status.

It is important to note that the overall ASD results presented in Table 2 are based on consensus of the study team after consideration of the ADOS scores in conjunction with other available measures including the SCQ, ADI-R, SRS, and application of DSM-IV and DSM-5 criteria to behaviors. Thus, for some participants, a change in ADOS diagnostic category between the ADOS and ADOS-2 did not change the overall consensus diagnostic impression of no ASD to ASD. In the participants who changed diagnostic classification from no ASD to ASD, applying the revised DSM-5 criteria to the participant's behavioral history was also important in supporting the change in diagnosis, especially in older patients where the DSM-5 criteria better explains manifestations of symptoms in adolescent to adult age groups, as well as due to the inclusion of sensory sensitivities as a component of the phenotype which are common in SCA. Overall, the percentage of the study group with a final classification of ASD increased slightly from 29.6% to 35.7% with the updated diagnostic tools; however, this difference was not statistically significant ($p = .44$).

Next, we examined ADOS-2 comparison scores between the 3 SCA groups as shown in Figure 2. Overall, mean autism symptom severity in boys with XYY and XXYY was higher compared with those with XXY/KS in both the no ASD and ASD groups. (No ASD: $F(2,55) = 4.20, p = .020$; ASD: $F(2,48) = 2.90, p = .046$). Scores in the no ASD group are expectedly lower compared with the ASD group; however, it is important to note slightly elevated mean scores in the no ASD groups as well. Within this no ASD group, 40% fell within the ASD or AUT range on one domain of the ADOS-2; however, overall scores or severity of symptoms were not supportive of a diagnosis.

To better understand the factors related to a diagnosis of ASD in the XYY and XXYY groups, we compared age, SES, prenatal versus postnatal diagnosis, cognitive factors (VIQ, PIQ, FSIQ), and adaptive scores between individuals with and without ASD as shown in Table 3. As there were no significant differences between the XYY groups at the 2 study locations, data from the 2 studies were pooled for the XYY analysis. XXY/KS was not included because of small sample size with ASD. Results showed that in both the XYY and XXYY groups, the group with ASD had significantly lower verbal IQ (XYY 84.6 vs 93.7, $p = .03$ and XXYY 68.9 vs 83.3, $p = .03$) and adaptive functioning scores (XYY 71.0 vs 91.0, $p = .009$ and XXYY 65.6 vs 81, $p = .008$) compared with the group without ASD. There were no significant differences in age, SES, PIQ, or FSIQ between the XYY groups with and without ASD. There was a lower proportion of ASD diagnoses in the prenatally diagnosed group (31%) compared with the postnatal diagnosis group (42%); however, this difference was not statistically significant ($p = .44$). As the entire XXYY group (ASD and no ASD) was diagnosed in the postnatal period, comparisons between features in prenatal versus postnatal diagnoses could not be made in this group.

DISCUSSION

This report describes study results from 2 sites evaluating autism spectrum disorder (ASD) in males with sex chromosome aneuploidy (SCA) using standardized autism assessment measures. In summary, results show that in 2 separate research cohorts of children with XYY, there were similar rates of ASD (approximately one-third of the group), and the SCA groups with Y chromosome aneuploidy (XYY and XXYY) were more likely to meet DSM-5 criteria for ASD compared with males with XXY/KS. The presence of ASD was associated with lower verbal cognitive abilities, and lower adaptive functioning scores. Compared with use of DSM-IV criteria and ADOS algorithms, use of DSM-5 criteria and revised ADOS-2 algorithms resulted in identification of slightly more individuals with SCA who met criteria for ASD; this difference was not statistically significant.

Previous studies have reported the rate of ASD in a sample of males with XYY to be 19% based on clinical history,[21] or 20% based on educational classification.[16] Our study builds on these results by directly assessing patients, using standardized autism measures, with very similar results between the 2 XYY cohorts and an overall rate of 38% for the combined group. Our rates may be slightly higher than previous studies because of identification of more ASD symptoms through the direct assessment, and also because of the change in ASD criteria

and increased ASD awareness and diagnostic rates in the general population over the past 20 years.

This study and the Geerts et al.[21] report (2003) compare the rates of ASD in prenatally versus postnatally ascertained cases of XYY, as this comparison is important in consideration of ascertainment bias and relevant to prenatal genetic counseling. Although our results show a slightly higher percentage of ASD in those with postnatal ascertainment (32% prenatal compared with 43% postnatal), this difference was not statistically significant. The Bishop et al.[16] study describes a rate of ASD of 20% in a prenatal XYY sample. Together, these results support that an elevated risk for ASD exists even within a prenatally diagnosed group with XYY. It is important to note that these reported rates of 20% to 32% are likely somewhat higher than would be expected from a true prenatal birth cohort, because there is still ascertainment bias (study participation bias) represented in the small samples evaluated for the research.

In comparison with previous studies of autism features in males with XXY/Klinefelter syndrome, Bruining et al.[19] (2009) evaluated 51 children with XXY/KS aged 6 to 19 using the ADI-R parent interview, with results showing that 27% met criteria for ASD. In our study, only 3 participants with XXY/KS with high SCQ scores received the ADI-R, and 1 of the 3 (33%) had a positive score. This rate of 33% is much higher than our final diagnoses of 5% to 10% obtained after a more comprehensive assessment including the ADOS/ADOS-2, DSM criteria, and team consensus. These discrepancies suggest that a more comprehensive assessment that includes direct evaluation of social interactions with the child using a measure like the ADOS may be important to differentiate between core social deficits of ASD versus many of the other neurodevelopmental differences associated with SCA such as language disorders or anxiety that may contribute to the ASD-like behaviors reported by parents on the ADI-R.

Another important finding in our study is that autism severity scores were elevated even in the SCA groups without ASD, and over one-third of SCA individuals without ASD met criteria in 1 domain of the ADOS. These results indicate that children with SCA can have a behavioral phenotype that overlaps with behaviors seen in ASD. Many of these overlapping behaviors, including language delays, poor eye contact, oversensitivity to sensory stimuli, poor social relatedness, and restricted interests, have been previously reported in children with SCA.[15,42] A 2008 study by Van Rijn et al.[15] that included 31 adults with XXY/KS reported significantly more distress during social interactions, less frequent social interactions, and more autistic traits including difficulties with communication, imagination, and social skills. This increased risk for ASD may be the result of similar underlying neurodevelopmental differences in core areas also known to be affected in SCA (including language, verbal cognition, and executive functioning). However, there is also evidence showing both neuroanatomical and functional MRI differences between males with XXY/KS and idiopathic autism, despite similar scores on clinical rating scales such as the SRS.[43,44] This suggests that at least some of the underlying neurobiologic abnormalities leading to clinical ASD symptoms may be different in XXY/KS. Future similar studies will also be important in other SCAs with higher reported rates of ASD such as XYY and XXYY. Further evaluation of other contributing genetic or environmental factors leading to a higher risk for ASD in all SCA subtypes is important.

From a clinical perspective, these results support that children with SCA are at increased risk for ASD, and that ASD should be considered when evaluating behavioral, social, educational, and emotional concerns. The neurodevelopmental features in some individuals with SCA can be complex and involve deficits in cognition/learning, language, and executive functioning that overlap with ASD features. Evidence-based treatments for ASD should be offered to children with SCA and ASD symptoms; however, studies are needed to determine if standard ASD treatments need to be modified for SCA. Many services and programs are well established in communities to support children with ASD and their families, and these services can potentially be advantageous for SCA as well.

Our study results support the fact that males with Y chromosome aneuploidy (XYY and XXYY) have an increased prevalence of ASD compared with males with XXY/KS and compared with XY males in the general population. This is reflected not only in higher rates of ASD in the XYY and XXYY groups, but also in the higher clinical severity scores in the groups without ASD, where the XYY group was higher compared with the XXY/KS group. The role of Y chromosome genes that contribute to the susceptibility of ASD and their mechanism in leading to neurodevelopmental changes are important for understanding the pathogenesis of ASD and may be very important in understanding the male predominance in ASD. There has been limited ASD research on Y chromosome–specific genes. Polymorphisms in the neuroligin 4Y (*NLGN4Y*) gene involved in neurodevelopment and synaptic function have been associated with ASD,[45,46] and Ross et al.[47] recently published genetic data demonstrating a positive correlation between *NLGN4Y* expression and ASD symptoms in children with XYY.

Another important Y-specific gene is SRY which is well known for its important role in triggering sexual differentiation. However, SRY is also expressed in human brain regions into adulthood and has been shown to directly modulate enzymes involved in both production and breakdown of important catecholamine neurotransmitters. More specifically, SRY has been shown to upregulate tyrosine hydroxylase (the rate-limiting enzyme in the synthesis of catecholamines dopamine, epinephrine, and norepinephrine), beta-endorphin production, and monoamine oxidase-A (MAO-A), the enzyme that breaks down monoamine neurotransmitters including the catecholamines and serotonin.[48–51] Polymorphisms in

MAO-A and abnormal levels of catecholamines, serotonin, and endorphin have been reported in various ASD studies and may thus be involved in the higher rates of ASD in XYY and XXYY as well.[52–54] Further investigation of the neurodevelopmental effects of Y chromosome genes is important as it will contribute to understanding both the male predominance in ASD and the increased rate of ASD in males with Y chromosome aneuploidy.

Ascertainment bias is a limitation in this study and an important consideration in all SCA research. Bias exists both in the ascertainment (diagnosis) of patients with SCA due to more severe clinical symptoms (thus in the general population those with more significant involvement are diagnosed), and in those who choose to participate in our research studies or who we reach through advertisements through support organizations. It is important to describe that in both studies, recruitment was not from a clinical sample, and recruitment flyers did not mention evaluation for ASD but a more general study of health and development. Although sample size and limited racial/ethnic diversity are additional limitations in this study, it is an important study in that it is the largest study to use and compare standardized ASD clinical assessment in a research cohort of males with SCA. Larger studies on a sample ascertained prenatally and followed prospectively are needed, and may soon be possible with recent advances in prenatal genetic testing which can identify SCA through genetic testing of maternal blood samples. This noninvasive prenatal testing has already started to notably increase diagnosis rates of infants with SCA.[55,56]

In summary, results support that ASD should be considered for males with SCA with social difficulties to help direct treatment recommendations, although other aspects of the phenotype including language and learning disorders, executive function, attention deficits, and emotional factors need to be considered during the diagnostic process and in developing a treatment plan. By comparing SCA subtypes, we have shown that Y polysomies are more likely to have autism compared with X chromosome polysomies, indicating the importance of future study of Y chromosome genes and Y chromosome gene dosage in potentially understanding the cause and male predominance of ASD.

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