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ORIGINAL PAPER

The Social Behavioral Phenotype in Boys and Girls with an Extra X Chromosome (Klinefelter Syndrome and Trisomy X): A Comparison with Autism Spectrum Disorder

Sophie van Rijn · Lex Stockmann · Martine Borghgraef · Hilgo Bruining · Conny van Ravenswaaij-Arts · Lutgarde Govaerts · Kerstin Hansson · Hanna Swaab

Abstract The present study aimed to gain more insight in the social behavioral phenotype, and related autistic symptomatology, of children with an extra X chromosome in comparison to children with ASD. Participants included 60 children with an extra X chromosome (34 boys with Klinefelter syndrome and 26 girls with Trisomy X), 58 children with ASD and 106 controls, aged 9 to 18 years. We used the Autism Diagnostic Interview, Social Responsiveness Scale, Social Anxiety Scale and Social Skills Rating System. In the extra X group, levels of social dysfunction and autism symptoms were increased, being in between controls and ASD. In contrast to the ASD group, the extra X group showed increased social anxiety. The effects were similar for boys and girls with an extra X chromosome.

Keywords Klinefelter · Trisomy X · Autism · Social functioning · X chromosome · Sex chromosomal aneuploidies

Introduction

Approximately 1–2 in 1,000 children is born with an extra X chromosome. In boys this leads to the 47,XXY karyotype (Klinefelter syndrome), and in girls to the 47,XXX karyotype (Trisomy X). These conditions have been associated with specific effects on physical, neurobiological, endocrinological and psychological development. Intellectual functioning is typically within the normal range, although slightly below average (for a review, see Boada

S. van Rijn (✉) · L. Stockmann · H. Swaab
Clinical Child and Adolescent Studies, Leiden University, Wassenaarseweg 52, 2333 AK Leiden, The Netherlands
e-mail: srijn@fsw.leidenuniv.nl

S. van Rijn · H. Swaab
Leiden Institute for Brain and Cognition, P.O. Box 9600, 2300 RC Leiden, The Netherlands

L. Stockmann
Autism Center Rivierduinen, Sandifortdreef 19, 2333 ZZ Leiden, The Netherlands

M. Borghgraef
Center for Human Genetics, University Hospital of Leuven, Herestraat 49, 3000 Leuven, Belgium

H. Bruining
Department of Psychiatry, University Medical Center Utrecht, Utrecht University, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands

C. van Ravenswaaij-Arts
Department of Genetics, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands

L. Govaerts
Department of Clinical Genetics, Erasmus Medical Center, Dr. Molewaterplein 50, 3015 GE Rotterdam, The Netherlands

K. Hansson
Department of Clinical Genetics, LDGA, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands

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et al. 2009; Leggett et al. 2010; Tartaglia et al. 2010b). Both boys and girls with an extra X chromosome are at elevated risk for deficits in speech, language, and communication development, and exhibit fine and gross motor impairments (Bishop et al. 2011).

Even though behavioral outcome in children with an extra X chromosome may be variable, there is empirical evidence suggesting that on average children (and adults) with an extra X have an increased vulnerability for social dysfunction. However, in contrast to the range of studies in boys and men (Klinefelter syndrome), social functioning in girls and women with an extra X (Trisomy X) has received much less attention. The reported social difficulties in boys and men include shyness, social withdrawal, social anxiety, difficulties in peer-relationships, social impulsivity, communication difficulties, impaired adaptive skills, reduced social assertiveness, emotion regulation problems and difficulties in reading social signals from others such as facial expressions, gaze direction and tone of voice (Bishop et al. 2011; Boone et al. 2001; Geschwind and Dykens 2004; Ratcliffe 1999; Ratcliffe et al. 1991; Robinson et al. 1991; Stewart et al. 1991; Tartaglia et al. 2010a; van't Wout et al. 2009; van Rijn et al. 2006, 2007, 2008; Visootsak and Graham 2009).

The severity of social difficulties in boys with an extra X chromosome is illustrated by the reported increased levels of autism symptomatology. Over 25 % of the boys with an extra X studied by Tartaglia et al. (2010a) showed autism traits as indicated by scores on the Social Responsiveness Scale that were in the mild-to-moderate or severe range as compared to norm scores (except for the subscale 'social awareness', with 20 % of the group showing abnormal scores). In adults with an extra X chromosome increased levels of autism traits, in areas of social skills, communication, attention switching and imagination, and increased attention to detail, have also been found (van Rijn et al. 2008). In some cases autism traits are substantially elevated and clinical criteria for diagnosis of autism spectrum disorder (ASD) are met. Bruining et al. (2009) reported that 14 out of 51 boys with an extra X chromosome (27 %) in their sample (a mixed group of referred cases and follow-up of cases determined prenatally) met the criteria for ASD, and in the study by Tartaglia (2010a) 1 out of 20 boys (5 %) met full criteria for ASD. Bishop et al. (2011) found that 2 out of 19 boys with an extra X chromosome (11 %) in their prenatal follow-up sample had a diagnosis of ASD.

Some studies have focused on social development in girls with an extra X chromosome, however many of these did not use standardized methods or control groups. Nonetheless, also in girls overall difficulties in social functioning seem indicated. In a longitudinal study by Harmon et al. (1998), the developmental transition from adolescence to adulthood was compared in 11 girls with an extra X chromosome and their siblings, using semi-structured interviews. This study revealed that girls with an extra X chromosome were less well adapted and had more relationship problems than female sibling controls. Shyness and social immaturity was observed in 8 out of 10 young girls with an extra X chromosome in a study by Robinson et al. (1991). Furthermore, Stewart et al. (1991) reported that 3 out of 6 girls with an extra X chromosome had difficulties with 'being sensitive and responsive to the feelings and rights of others'. A recent prenatal-follow up study by Bishop et al. (2011) showed that even though none of the girls with an extra X chromosome had ever received a diagnosis of ASD, they did have communication difficulties similar to those seen in ASD cases. The literature has been reviewed by Tartaglia et al. (2010b), who pointed to increased vulnerability for social avoidance, communication difficulties and social immaturity in many girls with an extra X chromosome. Another review (Otter et al. 2010) reported that many girls have difficulty in forming adequate interpersonal relationships, are shy, and show a lack of self-confidence. Bender et al. (1999) compared 36 boys and girls with an extra X chromosome, and their sibling controls, and found that boys as well as girls with an extra X chromosome showed more social problems than their siblings. Taken together, boys and girls with an extra X chromosome seem to be characterized by an increased risk for social dysfunction and a related risk for autism traits.

It is important to understand the type and severity of social behavioral problems in terms of autistic morbidity in children with an extra X chromosome. As identification of etiological pathways to psychopathology is a challenge because of the distant relationship between the clinical phenotype (the 'molar' level) and the underlying genotype (the 'molecular' level), there is a need for knowledge about the intermediary mechanisms along the genotype–phenotype pathway (Bearden et al. 2004; Gottesman and Gould 2003). Starting at the level of the genotype instead of the phenotype, i.e. reversing the typical line of research, may be a complementary approach in identifying such pathways. Studying genetic conditions such as children with an extra X chromosome (Klinefelter syndrome, Trisomy X) may lead to new insights into etiological pathways of autistic symptomatology. Importantly, individuals with an extra X chromosome are generally not intellectually disabled (in contrast to many other chromosomal disorders) which allows the study of the developmental mechanisms involved in social impairments, without the confounding aspect of general intellectual disability.

Although several studies have now reported an increased risk for autism spectrum traits in boys with an extra X chromosome, there has only been one study so far that has compared the social phenotype in these boys with to

children with ASD. Based on item scores from the Autism Diagnostic Interview Revised, Bruining et al. (2010) showed higher symptom homogeneity in boys with an extra X chromosome compared to autism, indicating that within the broad spectrum of autism symptoms a profile of symptoms was relatively specific for boys with an extra X chromosome.

We aimed to gain more insight into the social behavioral phenotype of children with an extra X chromosome compared to a psychiatric population of children with ASD. Our goal was to identify similarities and differences in social phenotype, by not only including measures of autism symptoms and traits, but also measures assessing general social skills and social anxiety. This allowed us to assess aspects of social development extending beyond the range of social behaviors that are typically affected in children with ASD. We were also interested in the degree of social difficulties in children with an extra X chromosome who did not show elevated levels of autism symptoms early in their development. Secondly, we aimed to contribute to the literature by comparing both boys and girls with an extra X chromosome to typically developing boys and girls. Although there have been several studies on social development in boys with an extra X chromosome, there are few neuropsychological and behavioral studies focusing on girls with an extra X chromosome.

Methods

Participants

In total, 60 children with an extra X chromosome (34 boys with Klinefelter syndrome and 26 girls with Trisomy X), 58 children (47 boys and 11 girls) with an autism spectrum disorder (ASD), and 106 non-clinical controls (46 boys and 60 girls) participated in the study. The participants were 9–18 years old. In the group of children with an extra X chromosome, we identified two subgroups. The first group included those families who were actively followed up after prenatal diagnosis with the help of clinical genetics departments. These departments of academic medical centers in the Netherlands and Belgium screened their databases for families who had received a prenatal diagnosis of Klinefelter syndrome or Trisomy X. Individuals in this group were considered 'prenatal follow-up' cases and constituted 53.3 % of the extra X group. Average maternal age was 48.1 (SD 4.4) years at the time of our study. The second group included those families who were actively seeking information about the condition of their child (recruited through support groups and calls for participants) and those who were seeking help for developmental problems (recruited through pediatricians, psychologists, psychiatrists, clinical genetics departments). These were considered 'referred' cases and constituted 46.7 % of the total extra X group. Average maternal age in this group was 47.6 (SD 6.4) years at the time of our study, which was not significantly different from the prenatal follow-up group ($p = 0.74$). These two different strategies allowed us to assess if our findings were affected by recruitment bias. Diagnosis of Klinefelter syndrome and Trisomy X was confirmed by standard karyotyping, all had non-mosaic kayotypes. In the group of boys, 28.1 % used testosterone supplements.

The ASD group was recruited from a child psychiatric outpatient department, serving a large region in the Netherlands. All children with ASD were classified according to the DSM-IV criteria (A.P.A. 1994). The clinical procedures for psychiatric assessment included questionnaires for parents, an interview with parents, developmental history and family history, information from treating physicians and extensive expert clinical observations. Consensus regarding the diagnostic classification of ASD had to be reached by board-certified child psychiatrists (with experience in the field of autism) and by a consensus meeting with a multidisciplinary team. In the ASD group, 41.1 % were diagnosed with autistic disorder, 30.4 % with Pervasive Developmental Disorder-not otherwise specified (PDD-NOS) and 28.6 % with Asperger's syndrome. Five out of 58 children with ASD were receiving psychopharmacological treatment.

Controls from the general population were recruited from schools distributed across the western part of The Netherlands. Children in the control group were screened for psychopathology: none scored in the clinical range (>70) on the Childhood Behavior Checklist (CBCL) (Achenbach 1991).

A Chi squared test indicated a significant difference (at $p < 0.001$) in the sex distributions between the groups: this could be attributed to a lower number of girls in the ASD

Table 1 Characteristics of the three groups, broken down by sex. Scores represent means and standard deviations

	Control group (n = 106)		Extra X group (n = 60)		ASD group (n = 58)	
	Girls (n = 60)	Boys (n = 46)	Girls (n = 26)	Boys (n = 34)	Girls (n = 11)	Boys (n = 47)
Age	11.7 (3.0)	12.3 (2.8)	11.6 (2.5)	13.8 (3.0)	12.7 (3.1)	11.8 (2.0)
IQ	104.6 (14.4)	101.5 (13.0)	78.0 (17.2)	79.9 (14.9)	110.0 (30.5)	99.7 (17.7)
Parental education level	2.1 (0.6)	2.1 (0.5)	2.2 (0.6)	2.3 (0.7)	2.5 (0.5)	2.4 (0.4)

group than in the other groups. MANOVA with the fixed factors group (control, extra X, ASD) and sex (boy, girl) and the dependent variables age, IQ and parental education, showed no significant main of effect of group, sex or group by sex interaction for age and parental education. However, there was a main effect of group (but no main effect of sex or sex by group interaction) for IQ, $F(2,193) = 29.6$, $p < 0.001$. Post-hoc LSD tests indicated this was driven by a significantly lower mean IQ in the extra X group as compared to both to control group and ASD group. Table 1 provides an overview of these variables.

Inclusion criteria for all participants were Dutch as the primary language and an age between 9 and 18 years. Exclusion criteria were a recent history of substance abuse, intellectual disability (<60 IQ points) and neurological conditions. These included structural brain damage due to prenatal/birth complications, traumatic head injury with loss of consciousness, tumors, stroke or infections, as well as neurological syndromes or diseases affecting the central nervous system. After providing a complete description of the study to the subjects and to their parents, we obtained written informed consent according to the Declaration of Helsinki. The study was approved by the Ethical Committee of Leiden University Medical Center, the Netherlands.

Early Autism Symptoms: ADI-R

The Autism Diagnostic Interview Revised (ADI-R) is a structured parent-report interview and widely recognized as the gold standard for establishing a clinical diagnosis of autism. The ADI-R is based on DSM-IV and ICD-10 diagnostic criteria for autism and generates algorithm scores for each of the three subdomains of autistic symptomatology; (a) qualitative impairments in reciprocal social behavior (b) deficits in language development and (c) restricted range of interest and/or stereotypic behaviors. For each domain a cut-off score is provided, above which a child meets the clinical criterion. We used the diagnostic algorithm, which is based on the (retrospective) functioning at age 4–5 years.

Current Autism Traits

The Social Responsiveness Scale (SRS) (Constantino and Gruber 2005) is a 65 item parent-report questionnaire that assesses the degree of autism spectrum symptoms as they occur in natural social settings. The SRS includes items that ascertain social awareness, social cognition, social communication, social motivation, and autistic mannerisms. Higher scores indicate stronger autism traits. A validation study (Constantino et al. 2003) indicated that the SRS was highly correlated with the ADI-R. Coefficients were higher than 0.64 between the SRS scores and all ADI-R scores. The Dutch version of the SRS has been validated and normed. T scores between 65 and 75 correspond to a 'mild or moderate' range of severity, and scores of 76 and higher are in the 'severe' range.

Social Anxiety

The Social Anxiety Scale (SAS) is a Dutch questionnaire to assess cognitive and affective reactions in four types of social situations: those in which social skills, intellectual skills, physical skills, and appearance are at stake. The fifth dimension measures social desirability. It is designed for children aged 8 and older. The reliability of the SAS is high (internal consistency = 0.90) (Dekking 1983), and its validity is satisfactory (Evers et al. 1992). The SAS consists of 36 items, which are each followed by two options: one option indicating social anxiety and the other indicating no social anxiety. For example: 'If someone in the group looks at me when I am doing something (1) I do not become nervous, (2) I become nervous'. Higher scores indicate more social anxiety.

Social Skills

The parent-report version of the Social Skills Rating System (SSRS) (Gresham and Elliott 1990) was used to assess social skills across four subscales: Cooperation (e.g. 'Helps you with household tasks without being asked'), Assertion (e.g. 'Starts conversations spontaneously rather than waiting for others to talk first''), Self-control (e.g. 'Ends disagreements with you calmly') and Responsibility (e.g. 'Requests permission before leaving the house'). Each of the four subscales consists of 10 items, which are rated on a 3-point Likert scale. Higher scores indicate better social skills. For the parent-report version of the SSRS, the internal consistency is 0.87 and the test–retest reliability 0.87. The Dutch version of the SSRS has been used in previous studies that were published in international journals.

Statistical Analyses

Data were analyzed using SPSS, version 19.0. Group effects on all behavioral measures were analyzed using MANCOVA, covarying for IQ. There were two fixed factors: 'group' (control, extra X, ASD) and 'sex' (boy, girl), with the number of dependent variables varying accoring the type of behavioral measure. LSD was used for post hoc group-wise comparisons. We analyzed the effect of recruitment bias, i.e. prenatal follow-up versus referred cases, on the measures in this study with ANOVA. The level of significance was set at $p \leq 0.05$. In case of significant differences, Cohen's d was used to calculate effect sizes.

Results

Recruitment Bias

Within-group analyses (ANOVA) comparing scores in the extra X group according to recruitment strategy, revealed no significant differences between 'prenatal follow-up cases' and 'referred cases', see Table 2.

ADI-R: Early Autism Symptoms in Children

Data from 3 participants in the extra X group and 9 in the ASD group were missing. MANCOVA (covaried for IQ) with the fixed factors 'group' and 'sex' and scores on the three ADI domains as dependent variables showed a main effect of group on ADI-R scores, $F(3,94) = 20.0$, $p < 0.001$. There was no significant main effect of sex or IQ and there were no significant group by sex interactions. Univariate results showed that ADI scores in the extra X group were significantly lower than in the ASD group in the Social interactions domain ($F(1,96) = 24.9$, $p < 0.001$, Cohen's d = 1.2), Communication domain ($F(1,96) = 36.2$, $p < 0.001$, Cohen's d = 1.2) and Stereotyped behaviors domain ($F(1,96) = 29.7$, $p < 0.001$, Cohen's d = 1.2). Mean score in the Social interaction domain was 11.6 (SD 8.3) in the extra X group and 18.5 (SD 4.2) in the ASD group. Mean score in the Communication domain was 10.3 (SD 5.1) in the extra X group and 15.3 (SD 4.3) in the ASD group. Mean score in the Stereotyped behaviors domain was 2.5 (SD 2.7) in the extra X group and 5.2 (SD 2.6) in the ASD group. For an overview of the percentage of children meeting ADI-R criteria, see Table 3.

Considering the absence of sex effects within the extra X and ASD groups, the data for boys and girls were not differentiated in further analyses of ADI scores. Based on the ADI-R data, the extra X group was subdivided into two subgroups, an ADI− group (n = 18) meeting criteria for 0 or 1 ADI domains and an ADI + group (n = 39) meeting criteria for 2 or 3 ADI domains (and thus similar to the ASD group). As the ADI-R scores were derived from the diagnostic algorithm, these data represent functioning at age 4–5 years. These subgroups were created for comparing measures in the study reflecting current functioning. The subgroups were similar with respect to age ($p = 0.55$), IQ ($p = 0.65$) and sex ($p = 0.61$).

Table 2 Age, IQ and social behavioral scores (mean, SD) in two subgroups of children with an extra X chromosome

	Prenatal follow-up group (53.3 %)	Referred group (46.7 %)	*p* values
Age (years)	12.5 (2.7)	13.3 (3.3)	0.30
IQ	77.7 (18.1)	79.8 (13.1)	0.63
Total ADI-R score (early autism symptoms)	22.9 (12.6)	25.3 (14.4)	0.49
Total SRS score (current autism traits)	64.0 (34.6)	69.0 (28.3)	0.58
Total SSRS score (social skills)	45.6 (13.3)	48.3 (12.4)	0.45
Total SAS score (social anxiety)	17.0 (12.2)	16.8 (12.7)	0.96

Table 3 Percentage of children with ADI-R scores above cutoff in the extra X group and ASD group

ADI-R domains	Extra X group (%)	ASD group (%)
Below cut-off on all domains	16.1	0
Above cutoff on 'Social interactions'	66.1	100
Above cutoff on 'Communication'	66.7	100
Above cutoff on 'Stereotyped behavior'	33.9	84.8
Above cutoff on two domains	48.2	19.6
Above cutoff on all three domains	19.6	80.4

Current Autism Traits

MANCOVA (covaried for IQ) with the fixed factors 'group' and 'sex' and scores on the SRS subscales as dependent variables showed a main multivariate effect of group on SRS scores, $F(10,380) = 22.3$, $p < 0.001$. There was no significant main multivariate effect of IQ, no significant multivariate effect of sex and there were no significant multivariate group by sex interactions. Considering that group effects were not dependent on sex, we combined the data from boys and girls in further analyses.

Univariate effects of group were significant on all SRS subscales at $p < 0.001$. Post-hoc group-wise comparisons showed that the extra X group scored significantly lower than the ASD group, but significantly higher than the control group on all subscales (all groupwise comparisons significant at $p < 0.01$), i.e. the extra X group scored in between the controls and the children with ASD. Total SRS score was 26.3 (SD 16.3) in the control group, 66.6 (SD 31.5) in the extra X group, and 97.6 (SD 28.8) in the ASD group. The effect size (Cohen's d) as compared to controls was 1.7 in the extra X group and 3.1 in the ASD group. Scores on subscales are presented in Fig. 1.

A separate MANCOVA comparing the ADI− and ADI+ groups with controls revealed a significant main multivariate effect of group ($F(10,286) = 11.8$, $p < 0.001$. Post hoc groupwise comparisons showed that the ADI+ group had significantly higher scores than the control group (at $p < 0.001$) and the ADI− group (at $p < 0.01$), on all SRS subscales. In other words, children with an extra X who had early autism symptoms, had increased SRS scores across

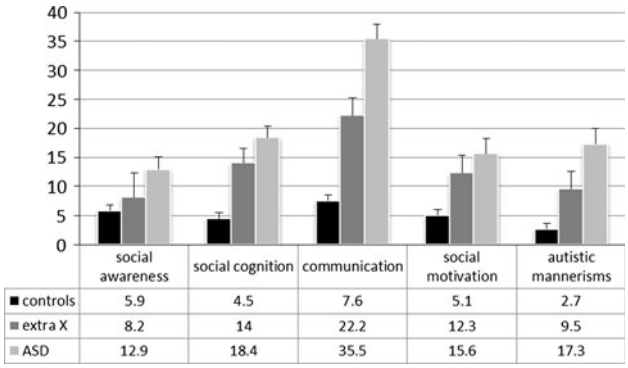


Fig. 1 Scores (mean, SD) on the subscales of the Social Responsiveness Scale in the extra X group, ASD group and control group. Higher scores indicate higher levels of autism traits

the board. Compared to the control group, the ADI− group also showed significantly higher scores on the 'social cognition' ($p < 0.001$), 'communication' ($p = 0.01$), 'social motivation' ($p = 0.006$) and 'autistic mannerisms' ($p = 0.04$) subscales. Only on the 'social awareness' subscale did they not show significantly higher scores than controls. In other words, on the SRS, children with an extra X chromosome who did not show early autism symptoms, still showed increased autism traits compared to controls. Within the extra X group, 55.8 % of the children had total SRS scores in the normal range, 19.2 % had total scores in the mild/moderate range (T > 65), and 25 % had total scores in the severe range (T > 75). See Table 4 for mean scores.

Social Anxiety

MANCOVA (covaried for IQ) with the fixed factors 'group' and 'sex' and scores on the SAS subscales as dependent variables showed a significant main multivariate effect of group on SAS scores, $F(12,398) = 2.6$, $p = 0.002$. There also was a significant main effect of sex $F(5,204) = 3.9$, $p = 0.002$, but no significant group by sex interactions ($p = 0.60$). In other words, girls in general showed more social anxiety, and this was similar for the control and extra X groups. Considering that group effects were not dependent on sex, we combined the data from boys and girls in further analyses.

At the univariate level, this was reflected in all individual subscales showing significant effects of group at $p < 0.005$. Post hoc group-wise comparisons revealed that the extra X group had higher SAS scores (i.e. more social anxiety) than controls on all the individual subscales, at $p < 0.001$. In contrast, scores in the ASD group did not significantly differ from controls, except for physical abilities which showed a slightly increased score ($p = 0.04$). The extra X group displayed more social anxiety than the ASD group on all five subscales: social interactions ($p = 0.004$), physical abilities ($p = 0.04$), intellectual abilities ($p < 0.001$), physical appearance ($p < 0.001$) and social desirability ($p < 0.001$). Total SAS score was 8.6 (SD 8.8) in the control group, 16.9 (SD 12.3) in the extra X group, and 10.5 (SD 9.8) in the ASD group. The effect size (Cohen's d) as compared to controls was 0.8 in the extra X group and 0.2 in the ASD group. Scores on subscales are presented in Fig. 2.

Table 4 Mean scores and standard deviations in children with an extra X chromosome who showed early autism symptoms (ADI+) and who did not show early autism symptoms (ADI−), as compared to the control group and children with ASD

	Controls	Extra X ADI-	Extra X ADI+	ASD
Total SRS score	26.3 (16.3)	49.4 (21.7)	73.9 (31.5)	97.5 (29.9)
Total SSRS score	60 (8.8)	57.2 (7.9)	43.1 (12.1)	35.9 (10.9)
Total SAS score	8.6 (8.8)	16.4 (10.8)	16.4 (12.9)	10.7 (9.8)

A separate MANCOVA comparing the ADI− and ADI+ groups with controls revealed a significant main multivariate effect of group ($F(10,296) = 4.0$, $p < 0.001$). Post hoc groupwise comparisons showed that both the ADI+ and ADI− groups had significantly higher scores than controls on all SAS subscales (at $p < 0.05$). The subgroups did not show significant differences in level of social anxiety. In other words, the extra X group showed increased social anxiety on all subscales, irrespective of the presence of early autism symptoms as measured with the ADI-R. See Table 4 for mean scores.

Social Skills

MANCOVA (covaried for IQ) with the fixed factors 'group' and 'sex' and scores on the Social Skills Rating

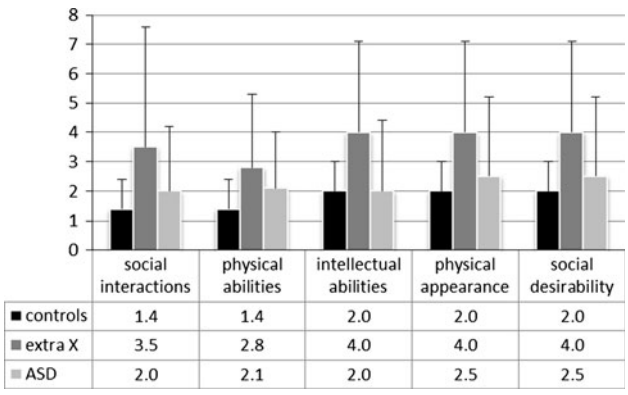
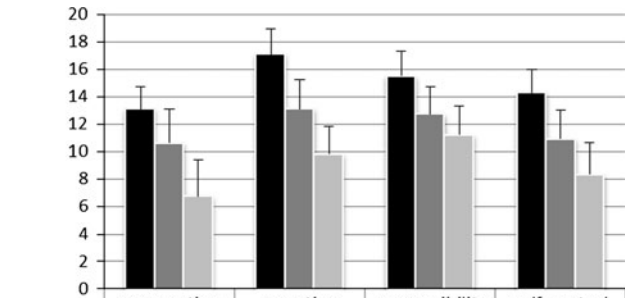


Fig. 2 Scores (mean, SD) on the subscales of the Social Anxiety Scale in the extra X group, ASD group and control group. Higher scores indicate higher levels of social anxiety

System subscales as dependent variables showed a significant main multivariate effect of group SSRS scores, $F(8,378) = 17.7$, $p < 0.001$. Univariate effects of group were significant on all SSRS subscales at $p < 0.001$. The post hoc groupwise comparisons showed that the extra X group scored significantly higher ('better') than the ASD group, but significantly lower ('worse') than the control group on all SSRS subscales (all comparisons significant at $p < 0.01$). In other words, the extra X group scored in between controls and children with ASD. Total SSRS score was 60.0 (SD 8.8) in the control group, 46.8 (SD 12.9) in the extra X group, and 36.3 (SD 11.0) in the ASD group, with higher scores indicating better social functioning. The effect size (Cohen's d) as compared to controls was 1.3 in the extra X group and 2.4 in the ASD group. Scores on subscales are presented in Fig. 3.

Furthermore, there was no significant main multivariate effect of IQ or sex, however there was a significant multivariate group by sex interaction $F(8,378) = 3.2$, $p < 0.001$. Univariate analyses indicated that this interaction was driven by the subscale'cooperation' ($p < 0.001$). Subsequent posthoc group-wise comparisons revealed that although boys with an extra X showed lower scores (i.e. more problems) in cooperation than control boys, girls with an extra X scored similar to control-girls.

A separate MANCOVA comparing the ADI− and ADI+ groups with controls revealed a significant main multivariate effect of group ($F(10,286) = 11.2$, $p < 0.001$). Post hoc groupwise comparisons showed that the ADI+ group had significantly lower scores than the control group (at $p < 0.01$) and the ADI− group (at $p < 0.01$) on all SSRS subscales. In other words, children who showed autism symptoms early in their development, had impaired social functioning across the board. In contrast, the ADI− group did not show significant differences to controls, on any of the SSRS subscales. In other words, children who did not show early autism symptoms, did not show deviant social functioning compared to controls on the SSRS. See Table 4 for mean scores.



	cooperation	assertion	responsibility	self control
controls	13.1	17.1	15.5	14.3
extra X	10.6	13.1	12.7	10.9
ASD	6.7	9.8	11.2	8.3

Fig. 3 Scores (mean, SD) on the subscales of the Social Skills Rating System in the extra X group, ASD group and control group. Higher scores indicate better social functioning

Differences Between Boys and Girls with an Extra X Chromosome

As we were particularly interested in comparing the social behavioral phenotype in boys and girls with an extra X chromosome, we explored this issue further. Although the multivariate analyses including the control, extra X and ASD groups did not point to substantial significant group by sex interactions, i.e. none of the measures (except for one subscale of the SSRS) showed significant group-by-sex interactions, we re-ran all analyses including only the control and extra X groups and excluding the ASD group which was characterized by a different sex distribution (more boys than girls). Excluding the ASD group did not change the pattern of results, as no significant group-by-sex interactions were found for the SRS ($p = 0.84$) and SAS ($p = 0.71$). Mean SRS score was 63.8 (SD 31.7) in girls with an extra X chromosome and 68.7 (SD 31.7) in boys with an extra X chromosome. Mean SAS score was 17.3 (SD 11.0) in girls with an extra X chromosome and 16.6 (SD 13.4) in boys with an extra X chromosome. Also, the total ADI-R score did not differ significantly between boys (24.3, SD 15.4) and girls (24.3, SD 11.8) with an extra X chromosome, $p = 0.99$. Again, only one subscale of the SSRS ('cooperation') showed a significant group-by-sex interaction ($p = 0.002$), in favor of girls with an extra X. Mean SSRS score was 47.8 (SD 12.1) in girls with an extra X chromosome and 46.1 (SD 13.6) in boys with an extra X chromosome.

Discussion

The aims of our study were to identify similarities and differences in social behavioral phenotype in children with an extra X chromosome and children with ASD and to examine similarities and differences in social functioning between boys and girls with an extra X chromosome. With regard to early autism symptoms, we found that 19.2 % of the children with an extra X chromosome met criteria (i.e. scored above cut-off) for all three ADI-R domains of autism symptomatology, using the diagnostic algortihm. Also, 48.2 % of the children scored above cut-off on two ADI-R domains. Although meeting ADI-R criteria is not equivalent to a clinical diagnosis of autism, the ADI-R is considered the gold standard clincial interview to measure autism symptoms by the field. Meeting all three criteria indicates serious risk, as this is typical for children with autism. Above cut-off scores on two domains of the ADI

(48.2 %) also points to substantial risk, as this is typically used in research studies as a means of describing children with PDD-NOS (Pervasive Developmental Disorder, Not Otherwise Specified) within the autism spectrum (Cox et al. 1999; Palmen et al. 2005; Sparks et al. 2002).

When considering dimensional measures of autism traits, as compared to the control group children with an extra X chromosome showed significantly increased levels of such traits across all the measured dimensions: social awareness, social cognition, social communication, social motivation, and autistic mannerisms. Within the extra X group, 19.2 % had total scores in the mild/moderate range ($T > 65$), and 25 % had total scores in the severe range ($T > 75$). Although their scores were significantly lower than those in children with ASD, these findings indicate that Klinefelter syndrome and Trisomy X can be associated with an increased vulnerability for autistic symptomatology. Rather than being restricted to a specific area of social or communicative deficit, there seems to be an increased risk for a range of autistic symptoms. With regard to general social skills, we also observed significant impairments in children with an extra X chromosome. More specifically, similar to children with ASD, the extra X group showed more difficulty in cooperating with others, being assertive in social situations, taking social responsibilities, and exerting self control in social situations than their typically developing peers.

Interestingly, there was one aspect of the social behavioral phenotype that discriminated between children with an extra X chromosome and children with ASD. In contrast to the ASD group, children with an extra X chromosome displayed increased levels of social anxiety compared to typically developing children. This was evident in situations involving social interactions as well as situations in which physical appearance, intellectual abilities or physical abilities were at stake, or in which social desirability played a role. Children with ASD only showed anxiety regarding their physical abilities. This is an interesting finding, as it suggests that children with an extra X chromosome differ from children with ASD in an important aspect, which is the ability to reflect on their own social functioning and to develop a related concern about social rejection, i.e. the opinions, thoughts and expressions of others. Maner and Kenrick (2010) argued from an evolutionary point of view that an optimal level of social anxiety can serve adaptive functions, such as to help people ensure an adequate level of social acceptance, but at high levels it can have harmful consequences such as excessive worry, negative affect, and exaggerated avoidance of social situations, leading to significant distress and social anxiety. According to Hofmann (2005), such anxiety is triggered when people perceive social situations as uncertain and uncontrollable. Hence, for many children with an extra X chromosome certain social situations may be unfamiliar and uncertain, and they may have difficulty predicting how others will behave. It will be interesting in future studies to assess the contribution of cognitive dysfunctions that may help explain why social interactions are more difficult. This may also be a relevant aspect to address in social behavior interventions for children with an extra X chromosome. Considering that these children may differ in their social reflective abilities compared to children with ASD, the approach taken in such interventions should probably be different.

Although this study was not longitudinal in nature, the use of the ADI-R algorithm which is based on retrospective functioning at age 4–5 years, helped us to form hypotheses about the characteristics of social development in children with an extra X chromosome. Splitting up this group into those who met at least two ADI-R criteria ('Social interactions' and 'Communication') at age 4–5 versus those who did not, allowed us to compare social functioning in children who did or did not show early autistic symptoms. This revealed that children who did not show high levels of autism symptoms at an early age, still went on to display significantly increased levels of autism traits and social anxiety compared to typically developing children. In fact, the level of social anxiety in this subgroup was similar to children with an extra X chromosome who did show autism symptoms at an early age. Thus, we speculate that, despite a lack of early autism symptoms, social dysfunction may become evident or more pronounced later on in the development of many children with an extra X chromosome. These findings are relevant from a clinical point of view, given that a substantial proportion of the children with an extra X chromosome have autism traits that may not exceed the threshold for a clinical diagnosis of autism spectrum disorder. These children may nonetheless be in need of clinical support, tailored to the profile of social behavioral problems typical for this group, i.e. high levels of social anxiety in addition to autism symptoms. From a clinical point of view, it may be important to note that a substantial proportion of the children with an extra X scored within the normal range on clinical measures of social functioning and autism traits. Within the extra X group, 55.8 % of the children had total SRS scores (measuring autism traits) in the normal range. Also, on the ADI-R, 16.1 % of the children scored below cut-off on all domains, indicating that there were no clinically relevant social difficulties, communication deficits or stereotyped/rigid behaviors.

We were also particularly interested in comparing the social behavioral phenotype between boys and girls with an extra X chromosome. Comparing the extra X group with the control group revealed a very consistent pattern of results indicating that none of the measures in this study

(except for one subscale) showed significant group by sex interactions. This was evident in levels of autism symptoms with the ADI-R, levels of autism traits as measured with the SRS, levels of social anxiety (SAS) and level of social skills of the SSRS (except for the subscale cooperation). In other words, the differences between girls with an extra X chromosome (Trisomy X) and their female peers were similar to boys with an extra X chromosome (Klinefelter syndrome) and their male peers. This finding also has clinical importance, since many of the findings in other studies on boys with an extra X chromosome might now also be extrapolated to girls with an extra X chromosome. In addition, it may have theoretical implications, specifically with regard to the etiology of social dysfunction in children with an extra X chromosome. For example, one could speculate that the shared social phenotype in boys and girls with an extra X chromosome could be (-partly-) attributed to shared genetic mechanisms. We hope that our findings stimulate future genetic studies on this issue. The fact that neurodevelopment and social skills are for a large part driven by genetic factors, possibly as high as 68 % for social skills (Scourfield et al. 1999), warrants further study of genetic conditions such as Trisomy X and Klinefelter syndrome Such X chromosomal aneuploidies are particularly interesting, given the exceptionally high density of genes on the X chromosome that are essential for neural development (Zechner et al. 2001), the diagnosis on genetic level allowing early identification (as early as prenatally), and related bottom-up approach in the study of social behavioral development.

In our study, the social behavioral phenotype was not dependent on recruitment strategy (i.e. prenatal follow-up cases or referred cases), which suggests that our findings are representative for this group of diagnosed children as a whole. In the Netherlands, the standard clinical guidelines are that women above the age of 36 are offered prenatal screening at no additional cost. As this group was traced with the help of the clinical departments where karyotyping was performed 8–19 years ago, this group may represent a broad spectrum of outcomes. However, one cannot exclude that increased and early awareness of behavioral problems may have affected social behavioral outcome. Although referred cases were also included, i.e. families who were actively seeking information about the condition of their child (recruited through support groups and calls for participants) and those who were seeking help for developmental problems (recruited through pediatricians, psychologists, psychiatrists, clinical genetics departments), to capture the full spectrum of behavioral outcome, there was no systematic difference in behavioral outcome detected by our study. As maternal age was similar across the subgroups, this was also not a confounding factor. Nonetheless, it remains unsure to what degree the findings in this study can be extrapolated to those who have an extra X chromosome but remain undiagnosed, which is a considerable proportion of of the individuals. One can merely speculate about the reason why some individuals with an extra X chromosome are not ascertained. One may argue that the phenotype is milder in those that remain undiagnosed. However, one could also speculate that individuals with an extra X chromosome may be treated for behavioral problems, without knowing about their underlying chromosomal pattern.

This study had some limitations, such as differences in the level of intellectual functioning. Although the ASD group had a mean IQ level similar to the control group, the mean IQ level was significantly lower in the extra X group. However, as all statistical analyses were corrected for IQ, this could not explain the differences observed in social behavioral phenotype. The number of girls in the ASD group was also small, which may have reduced the power to find sex effects in this group. However, the main interest was in sex effects within the extra X group. Furthermore, analyses included subscale scores, rather than single item scores as reported by Bruining et al. (2010), which did not allow identification of profiles on such a detailed level. This may also have contributed to differences in outcome, i.e. this study showed that the overall range of ASD symptoms were increased in children with Klinefelter or Trisomy Xs, whereas Bruining et al. showed that children with Klinefelter syndrome could characterized by a specific profile of autism symptoms. Finally, children in the ASD and control group were not screened for having an extra X chromosome. As the estimated prevalence of XXX/XXY in the general population (about 1 in 500–1,000) and the ASD population (1 in 127 in the study of Konstantareas and Homatidis 1999), we may have included an undiagnosed child with an extra X in each of these groups. Considering the effect sizes found, this could not have significantly affected our findings.

All in all, this study has illustrated the importance of understanding how individual differences contribute to social dysfunction, how this may help to identify children who are likely to benefit from specific interventions, expanding our understanding of the specific neuropsychological processes that underlie social dysfunction in different children. Future studies in this field are in need of a cognitive approach that may help uncover the neuropsychological mechanisms driving the social behavioral phenotype in individuals with an extra X. For example, it would be interesting in future studies with children with an extra X chromosome to study social perception, which has received much attention in studies on social anxiety. There is already evidence that men with an extra X chromosome are at risk for deficits in interpreting facial expressions (van Rijn et al. 2006), affective tone of voice (van Rijn et al.

2007) and gaze direction (van't Wout et al. 2009). Such studies may help improve understanding of the different developmental pathways to risk for ASD and possibly identify subgroups within the broad ASD spectrum that are in need of different intervention strategies.

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Conflict of interest The authors declare that they have no conflict of interest.

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