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The Effect of Sex Chromosome Number Variation on ADHD Symptoms, Executive Functions, and Processing Speed

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Abstract

Aim: **Offer a new paradigm** to the study of sex differences in attention deficit hyperactivity disorder (ADHD) symptoms, we directly test, in humans, whether X chromosome absence or excess is independently associated with deficits in attention and hyperactivity, executive function, and processing speed.

Methods: We assessed 114 children (ages 3.8–11.9 years) with a variable number of sex chromosomes: 36 girls with Turner syndrome (45, X0), 20 boys with Klinefelter syndrome (47, XXY), 37 typically developing girls (XX), and 23 typically developing boys (XY).

Results: X chromosome absence was associated with increased attention problems, hyperactivity, and deficits in inhibitory control, compared with girls with XX (all p-values<0.003). Conversely, X chromosome excess was associated with weakness in working memory (p=0.018) and approached significance for attention problems (p=0.071) but not with hyperactivity, or weakness in inhibitory control relative to boys with XY. Using **non-parametric** effect size to quantify the clinical effect revealed that X chromosome absence affected attention, hyperactivity, executive function, and processing speed (**all *r*>0.4**) while X excess affected **in-lab as well as parent reported** working memory (**all *r*>0.4**).

Conclusion: Our observations provide compelling evidence that the absence or excess of X chromosome distinctly affects cognition and behaviors associated with ADHD.

What this paper adds:

- X chromosome number has a distinctive effect on ADHD symptoms.
- X chromosome absence is associated with increased attention problems, hyperactivity, and weakness in executive functions.

- X chromosome excess is associated with weaknesses in attention and working memory.
- Clinical management for children with Turner syndrome should focus on attention problems, hyperactivity, and weak inhibition skills.
- Clinical management for children with Klinefelter syndrome should focus on attention problems and impaired working memory.

Introduction

Sexual dimorphism in neurodevelopmental disorders, such as autism spectrum disorder and attention deficit hyperactivity disorder (ADHD), has been widely recognized. For ADHD, differences between males and females in prevalence, course, comorbidities, and clinical manifestations are hallmarks of this diagnosis. Concerning clinical manifestations, girls with ADHD are more likely than males to be diagnosed with the predominantly inattentive type of ADHD (1), while symptoms severity is higher in males compared to females (2). The complex array of genetic, hormonal, and social differences associated with ADHD obscures the biological factors affecting sexual dimorphism in this disorder (3) Not surprisingly, traditional methods comparing males and females with idiopathic ADHD to controls have yielded limited information about the relative contribution of biological factors to observed behavioral differences between the sexes (4). The study of sex chromosome aneuploidies (SCA), which are associated with changes in sex chromosome number and constitution, has emerged as a promising strategy for elucidating genetic, hormonal and **inflammatory** substrates of sexual dimorphism in the manifestation of ADHD **symptoms** (3,5). **This approach of studying children with sex chromosome aneuploidies is aimed to simplify the investigation of complex behaviors such as attention and hyperactivity in humans.**

Two common SCAs are Klinefelter syndrome (KS) (most commonly a 47, XXY karyotype; one in 500 male live births) (6), and Turner syndrome (TS) (most commonly 45, X0; one in 2000 female live births) (7). In typically developing (TD) girls (XX), one of two X chromosomes undergoes inactivation, yet about 15% of inactivated X chromosome genes escape inactivation, resulting in expression of two gene copies; only one gene copy is present in girls

with TS. Genes on the additional X chromosome in KS (47, XXY) also escape inactivation, resulting in increased X chromosome gene expression in boys with KS in comparison to TD boys, along with Y chromosome gene expression.

TS and KS present inverse profiles of strengths and weaknesses in many cognitive dimensions in the context of overall normal intelligence (8–10). While girls with TS present with relative weakness in visuospatial abilities and strengths in verbal skills, boys with KS usually present with relative strength in visuospatial abilities and weaknesses in verbal skills. Nevertheless, both SCAs have been associated with weaknesses in social cognition (11–14) and executive function (EF) (9,15–17), and ADHD symptoms (16,18–20). While several studies show that females with TS present with weaknesses in processing speed (21), KS data for males are less conclusive. Some studies show no differences compared to TD controls (22). Others find weaknesses in processing speed, with some evidence for more impairment in verbal rather than non-verbal information processing (15).

With respect to ADHD symptoms, 25% of girls and adolescents with TS present with an extensive set of related EF weaknesses (23,24) and meet Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria for ADHD (25), while we reported in our previous study (15) that half of a distinct cohort of girls with TS displayed significant levels of attention problems and hyperactivity. Using a national registry, 4.9% of individuals with KS had registered or received treatment for an ADHD diagnosis (26). In contrast, children with KS had a 63% rate of ADHD diagnosis using the Kiddie-Sads-Present and Lifetime Version (K-SADS-PL) interview (27). Use of the Conners' Parent Rating Scale–Revised-Long Version indicates that 42% of boys with KS have significantly elevated rates of ADHD compared to TD boys (28).

Similarly, using the DSM-IV, 36% of males with KS meet diagnostic criteria for ADHD, with predominantly inattentive symptoms (29).

Given previous reports of significantly elevated risk for ADHD symptomatology in TS and KS, we hypothesized that both SCAs would be associated with an overall increase in ADHD symptoms in the new cohort described here. However, based on our previous results outlining elevated levels of hyperactivity in girls with TS compared to girls with non-syndromic ADHD (16), we further predicted that differences in X chromosome number would be associated with distinct ADHD symptom profiles for TS and KS. Specifically, we hypothesized that TS (45, X0) would be associated with a 'male ADHD profile' (i.e., increased attention problems, hyperactivity, and EF weaknesses), while KS (47, XXY) would be associated with a 'female ADHD profile' (i.e., predominantly increased attention problems, along with a corresponding pattern of EF weaknesses).

To test our hypotheses, we compared girls with TS and boys with KS to their respective TD sex-matched controls on a comprehensive assessment of ADHD symptoms and associated cognitive measures of EF. Also, we contrasted each SCA with the opposite-sex TD controls to provide additional information about the effects of sex chromosome number and constitution (and specifically X chromosome number) on cognition and behavior. To the best of our knowledge, such a direct assessment comparing ADHD profiles among girls with TS, boys with KS, and TD controls has not been conducted to date.

Methods

Participants

One-hundred fourteen participants (ages 3.8–12 years) were included in this study: 36 girls with TS and 20 boys with KS were recruited through pediatric endocrinologists, medical geneticists, the national Turner Syndrome Society network, the Association for X and Y Chromosome Variations, and the Center for Interdisciplinary Brain Sciences Research website. Thirty-seven TD female and 23 male controls were recruited through local parent organizations, advertisements, and from siblings of TS or KS participants. More information about the participants can be found in the supplementary materials.

This study was approved by the local Institutional Review Board of the Stanford University School of Medicine, and informed written consent was obtained from a legal guardian for all participants, as well as written assent from participants over 7 years of age.

Study Design

The four groups comprising this study were compared on behavioral measures of attention and hyperactivity using the Behavior Assessment System for Children, Second Edition (BASC-2), specifically Hyperactivity and Attention Problems, and on cognitive-behavioral measures of EF. Cognitive and behavioral measures of EF included the Behavior Rating Inventory of Executive Function (BRIEF), specifically Inhibit, Shift, Emotional Control, Working Memory, and Plan/Organize the NEuroPSYchological test, version 2 (NEPSY-2), specifically Auditory Attention, Response inhibition, Naming, Inhibition and Switching, and the Wechsler intelligence test working memory (Digit Span and Letter Number Sequencing) and

processing speed Coding and Symbol Search) subscales. Details on measures can be found in the supplementary materials.

Data analysis

For all analyses, we use the R software for statistical computing version 4.0.2. We employed non-parametric tests given the non-normal distributional properties of the data (particularly the male groups). Group ages and Full Scale IQ (FSIQ) scores were compared with the Kruskal-Wallis test. The Fisher's exact test was employed to compare pubertal development (31), represented by Tanner scores for pubic hair and female breast/male genitalia development. Due to the low frequencies of participants with Tanner score >2, we combined participants in Tanner stages 3 (n=7) and 4 (n=2) into one group (Table 1).

We used the Kruskal-Wallis test to compare attention problems, hyperactivity, and cognitive measures of EF between groups. For measurements with significant Kruskal-Wallis test results, the Wilcoxon rank-sum (**also named Mann–Whitney *U* test**) test was used to conduct post hoc analyses comparing the study group pairs. BRIEF T-scores are already adjusted for sex, thus, for this measurement we only compared same-sex group pairs (i.e., TS vs. TD girls and KS vs. TD boys).

Holm's adjustment (30) was used to control for multiple comparisons for each cognitive-behavioral outcome category (instrument), i.e., BASC-2, BRIEF, NEPSY, and IQ subtests.

Finally, to measure the clinical effect of sex chromosomes on cognition and behavior, we used between subject, non-parametric effect sizes r using the rank-biserial correlation (specifically, we used the r -function effectsize::rank_biserial)(31)**). This rank is calculated from the Wilcoxon rank-sum test: a non-parametric statistical test used to compare two**

non-paired groups. The rank-biserial correlation is calculated using average ranks from two sets of data and sample size in each group. To interpret the calculated value, one can draw on the interpretation of the classical Pearson's correlation coefficient (r), hence the strength of the relationship (31).

Results

Demographics

Study groups did not differ significantly in age (Kruskal-Wallis χ^2 (3)=1.19, p=0.76; Table 1), or pubertal development (Fisher's exact test, p=0.22; Table 1). As expected (11,12,32), FSIQ differed significantly between groups (Kruskal-Wallis χ^2 (3)=43.2, p<0.001), with both SCAs receiving comparably lower FSIQ scores compared to sex-matched TD controls. However, it should be noted that all four groups demonstrated a mean FSIQ within the range of average intelligence (Table 1, Figure S1).

Behavioral measures of ADHD symptoms

The four study groups differed in behavioral measures of attention problems and hyperactivity (Table 2; Figure 1). **Post hoc** analysis revealed that girls with TS had elevated parent-reported attention problems and hyperactivity compared to TD girls. Overall, boys with KS had elevated parent-reported attention problems but not hyperactivity compared to TD boys. Using a cut-off of 60 and above in the BASC-2 assessment (scores above the "at-risk" level for the measured behavior), we observed that 42% of girls with TS were reported by their parents to have attention

problems and hyperactivity. Fifty-five percent of boys with KS were parent-reported as having attention problems while 40% had hyperactivity (Table 1).

Executive function – parent-reported and in-person lab evaluation

In general, we observed that girls with TS scored higher (i.e., were more impaired) than TD females on most of the parent-reported EF metrics. Boys with KS scored higher only on Working Memory compared to TD boys (Table 2, Figure 1).

The four study groups differed significantly in cognitive tasks of **continues** attention and inhibition (Table 2, Figure 2). Post hoc analyses revealed that girls with TS performed worse on **continues** attention and inhibition tasks than TD controls.

Notably, across all groups, 24 participants were not within the required age range (>7 years) for the Response Inhibition and Switching NEPSY-2 subtests. However, the percentage of participants who did not complete this task did not differ between groups (χ^2 (3)=5.91, p=0.12; see supplemental material for the complete number of participants who completed each subtest of the NEPSY-2 and other tests administered).

We further evaluated processing speed across the entire age range (3.8–11.9 years) using processing speed subtests from the IQ measures. We observed differences between study groups for Coding and Symbol Search (Table 2; Figure 2); post hoc analyses revealed that girls with TS scored lower than TD girls. Conversely, scores from boys with KS were generally comparable to those of TD boys on tasks measuring processing speed (Coding and Symbol Search) though were lower compared to TD girls for Coding (p=0.026).

Finally, SCA groups differed from controls on working memory subtests from the IQ measures (Table 2; Figure 2). Scores of girls with TS and boys with KS were lower than those of TD controls of both sexes for the Digit Span and Letter-Number Sequencing subtests.

The effects of X chromosome number on cognition and behavior

To measure the clinical effect of sex chromosome variation on ADHD symptoms and EF, we calculated effect sizes and confidence intervals for all measures significantly different between the groups (Figures 1 and 2). We observed significant effects (confidence interval does not cross 0) of TS and KS on parent reported as well as in lab measures of working memory (parent-reported Working Memory, Digit Span Letter Number Sequencing) compared to same-sex control groups (**Figure 3**). In contrast, TS but not KS had a significant effect on attention and hyperactivity (parent-reported Attention problems and Hyperactivity, **Auditory Attention**) and broad-ranging problems with executive functions including inhibition (parent-reported Inhibit and Inhibition), planning (parent-reported Plan/Organize), shifting (parent-reported Shift and Response Set), processing speed and working memory (Coding, Symbol Search, Digit Span and, Letter Number Sequencing). These results suggest that while children with SCAs have ADHD symptoms and associated weaknesses in executive function compared to controls, each SCA **has** a distinct profile of ADHD symptoms and related executive function weaknesses.

Discussion

Our findings show that girls with TS and boys with KS display increased ADHD symptoms and weaknesses in EF relative to TD controls. Further, we find distinct behavioral and cognitive profiles in these genetic conditions. Compared to female and male control groups, girls with TS display elevated levels of attention problems, hyperactivity, and weaknesses in

inhibition skills. Boys with KS show attention problems and working memory weaknesses compared to female and male control groups. **Using effect sizes, we further confirm the clinical significance of our results, demonstrating significant effect sizes for the TS group for attention problems, hyperactivity, and weaknesses in auditory attention, inhibition skills, and processing speed. For both SCAs we find significant effect sizes for parent-reported working memory and in lab measures of working memory compared to same sex-controls (Figure 3).**

Our findings are mostly in line with previous studies of ADHD symptoms (15,22,25–28) and EF (8,9,15,24) in individuals with TS or KS. For girls with TS, an increase in attention problems and hyperactivity has been well documented (15,22). The observed pattern of ADHD symptoms (i.e., increased attention problems without hyperactivity) among boys with KS is also in line with previous studies in young boys (<10 years) with KS (7,30). The present study results expand the previous framework of ADHD in each of the syndromes by finding a distinctive profile of ADHD symptoms in TS and KS when tested simultaneously.

Similarly, we observed a distinct EF profile in TS and KS. In TS, affected domains included working memory, processing speed, and inhibition abilities. These results are consistent with earlier studies reporting weaknesses in these EF domains using the NEPSY(12,16), BRIEF (23), and Rey Figure Organizational score (33), as well as tasks such as the Contingency Naming Task (34) and the Stroop Test (35) measuring inhibition skills. **In contrast, in KS, affected domains included parent-measured and in-lab working memory, but not in processing speed or inhibition skills (Figure 3).** The deficits in inhibition in TS are aligned with the broader literature of ADHD in females showing a link between performance in response inhibition tasks and hyperactive-impulsive symptoms (36). The deficits in parent-reported

working memory measures are aligned with the cognitive measures of working memory (assessed using the Digit Span and Letter-Number Sequencing subtests) in both groups. Yet, it should be noted that these measures also tend to be lower in children with reading impairments, a common finding in KS (37,38). Thus, for KS, we cannot conclude whether these findings are specifically secondary to learning impairments or attention problems. Overall, the observation of inhibition deficits in TS (45, X0) but not in KS (47, XXY) suggest a protective effect of the X chromosome in this domain.

The number of X chromosomes is thought to affect neurodevelopment through various mechanisms including genes that escape X inactivation, the pattern of random X inactivation in females, parental X imprinting, and epigenetic effects (39). Furthermore, the number and constitution of sex chromosomes may also have a more widespread effect on genome-wide gene expression (40). X chromosome genes that escape inactivation and lack a Y chromosome homologue are potential candidates for sexual dimorphism (41) due to the resulting variable gene dose in males and in females. The loss of genes that escape inactivation in TS is suggested as a possible explanation for the susceptibility of girls with TS to develop ADHD symptoms and EF weaknesses (15). A recent study (41) examining gene expression in SCA confirmed the haploinsufficiency of genes that escape inactivation in TS. Raznahan et al. (41) found that inactivated X-linked genes are overexpressed in TS yet undergo further silencing with mounting numbers of X chromosomes (XXY or XXX or XXYY). Therefore, it is predicted that males with KS who have two X chromosomes are theoretically more likely to phenotypically mimic females with idiopathic ADHD. The results of the present study support this hypothesis. The distinct ADHD symptoms and EF profile found in the focal SCAs provide a potential biological explanation for the male-predominance in early neurodevelopmental disorders, including ADHD

(4) and the relatively high rates of ADHD symptoms of attention problems and hyperactivity in girls with TS.

Caution should be exercised when interpreting results of this study due to several limitations. Our TD boys and KS groups were smaller than TD girl and TS groups, probably contributing to the result that many of the KS versus TD boys comparisons yielded only a near-significant trend. Effect size analyses allowed us to partially overcome this limitation (42). For the KS group, we include limited data on the timing of diagnosis (Table 1). Unlike TS, which is associated with a physical phenotype that leads to syndrome detection, children with KS might not present an apparent phenotype indicating the condition. Prior work has shown that the degree of impairment faced by youth with sex chromosome trisomies, including KS, is correlated with the timing of diagnosis (either pre- or post-natal) (43). Another limitation of this study is that although comparisons in pre-pubertal individuals with SCAs provide a rather lucid examination of the effects of changes in sex chromosome number and constitution, changes in hormonal profile (especially in TS) probably also affect neurodevelopment (44). **On processing speed measures, one should consider that the WISC processing speed index has a large motor component – particularly the Coding subtest. Thus, the motor difficulties that are known to be associated with TS** (45) **and KS** (18) **could be contributing to the reduced processing speed observed in TS.** Finally, given our relatively small sample size of the male groups and our choice to use non-parametric testing, we are not including IQ as a covariate in our group comparisons. This approach potentially limits our ability to test whether group differences in ADHD symptoms and EF are derived from SCA genotype only or also by overall cognitive abilities.

In conclusion, we herein expand the framework for examining the effect of X chromosome number on ADHD symptoms and associated cognitive constructs using two SCAs, enabling unique contrast of X chromosome absences vs. excess. Our results stress the association between X chromosome number and sexually dimorphic presentation of attention, hyperactivity, and EF, and inform clinical management in these common SCAs. We suggest that specific treatments and outcome measures targeting symptoms of hyperactivity and inhibition skills should be utilized in clinical management of ADHD symptoms in TS. For KS, observations from the present study and others (18) suggest that boys with KS have increased tendency for attention problems without hyperactivity, and therefore are more likely to be underdiagnosed and undertreated (similarly to girls with idiopathic ADHD (46)). Thus, increased vigilance for symptoms of attention problems in KS may affect long-term outcomes in individuals with this common genetic condition.

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Table 1. Demographics

	TS	KS	TD girls	TD boys
Number of Participants	38	20^	38	23
	Mean (SD)	*Mean (SD)*	*Mean (SD)*	*Mean (SD)*
Age	8.4(1.9)	8.4(2.2)	8.8(1.6)	8.0(2.2)
Age Range	3.81-10.8	4.72-11.2	4.89-11.7	3.95-11.9
FSIQ	93.2(12.7)	96.5(11.2)	111.0(10.1)	111.3(8.4)
PSI/PIQ	91.8(14.7)	103.6(13.6)	112.8(11.6)	113.1(11.2)
VCI/VIQ	105. 4(13.9)	97.2(12.1)	111.6(13.1)	116.0(14.4)
PSI/PSQ	82.4 (15.2)	92.1 (10.4)	100.2 (11.2)	97.0 (12.0)
WMI	90.0 (10.5)	89.1 (14.0)	103.7 (10.1)	102.0 (9.0)
Attention Problems	16 (42.1%)	11 (55%)	5 (13%)	2 (8.6%)
Hyperactivity	16 (42.1%)	8 (40%)	2 (5%)	2 (8.6%)
Medications				
GH	31 (81.6%)	---	---	---
Sex hormones	1 (0.025 mg estrogen patch)	1 Oxandrolone	---	---
Stimulants	---	---	---	---

Tanner Stage	Pubic Hair *N (%)*	Breasts *N (%)*	Pubic Hair *N (%)*	Male Genitalia *N (%)*	Pubic Hair *N (%)*	Breasts *N (%)*	Pubic Hair *N (%)*	Male Genitalia *N (%)*
1	35(92.1)	34(89.5)	19(100)	16(84.2)	26(72.2)	26(72.2)	21(95.5)	19(86.4)
2	1(2.6)	4(10.5)	0	2(10.5)	7(19.4)	9(25)	1(4.5)	1(4.5)
3	2(5.3)	0	0	1(5.3)	2(5.6)	0	0	2(9.1)
4	0	0	0	0	1(2.8)	1(2.8)	0	0
5	0	0	0	0	0	0	0	0

Note: girls with Turner syndrome (TS), boys with Klinefelter syndrome (KS), typically developing girls (TD girls), and typically developing boys (TD boys), participants using growth hormone (GH), Full Scale Intelligence Quotient (FSIQ) from the Wechsler Preschool and

Primary Scale of Intelligence Third Edition (WPPSI-III) or Wechsler Intelligence Scale for Children Fourth Edition (WISC-IV), composite scores for the Perceptual Comprehension Index from the WISC-IV or Performance Intelligence Quotient from the WPPSI-III (PRI/VIQ), Verbal comprehension Index from the WISC-IV or Verbal Intelligence Quotient from the WPPSI-III (VCI/VIQ), Working Memory Index from the WISC-IV (WMI), and the Processing Speed Index from the WISC-IV (PSI/PSQ).

^ Of the KS cohort, two participants were diagnosed prenatally, seven postnatally, and for 11 participants, these data were not available.

Table 2. Summary statistics

	KW χ^2 (*p* value)	TS vs TD girls *p* value	TS vs TD boys *p* value	KS vs TD girls *p* value	KS vs TD boys *p* value
Attention Problems	21.43 (<0.001)	<0.001	<0.01	<0.01	0.07
Hyperactivity	17.98 (<0.001)	<0.001	<0.01	<0.05	0.26
Inhibit	--	<0.05	--	--	0.22
Shift	--	<0.05	--	--	0.08
Emotional Control	--	0.25	--	--	0.06
Working Memory	--	<0.001	--	--	<0.05
Plan/Organize	--	<0.05	--	--	0.07
Auditory Attention	7.88 (<0.05)	<0.05	<0.05	0.22	0.15
Response Inhibition	11.14 (<0.05)	<0.01	p<0.05	0.07	0.24
Naming	0.19 (0.34)	0.19	0.99	p<0.05	0.28
Inhibition	17.93 (<0.001)	<0.001	<0.01	0.13	0.19
Switching	2.78 (0.43)	0.14	0.26	0.47	0.61
Digit Span	21.57 (<0.001)	<0.001	<0.001	<0.01	0.05
Letter- Number Sequencing	26.67 (<0.001)	<0.001	<0.01	<0.001	<0.001
Coding	20.89 (<0.001)	<0.001	<0.05	<0.05	0.25
Symbol Search	23.71 (<0.001)	<0.001	<0.001	0.67	0.55

Note: Kruskal-Wallis (KW), girls with Turner syndrome (TS), boys with Klinefelter syndrome (KS), typically developing girls (TD girls), and typically developing boys (TD boys)

Figure 1.
Title: Behavioral symptoms of ADHD and executive functions in girls with Turner syndrome, boys with Klinefelter syndrome, and typically developing girls and boys.
Caption: Behavioral assessment of ADHD symptoms and executive function profile for girls with Turner syndrome (TS), boys with Klinefelter syndrome (KS), typically developing girls (TD girls), and typically developing boys (TD boys). Behavioral symptoms of ADHD were measured using the Behavior Assessment System for Children (BASC-2) Attention Problems and Hyperactivity scales. Executive function profile was measured using the attention and executive function scales/subtests of the Behavior Rating Inventory of Executive Function (BRIEF) scales: Inhibit, Shift, Emotional Control, Working Memory, and Plan/Organize.

Figure 2.
Title: Detailed Cognitive profile for girls with Turner syndrome, boys with Klinefelter syndrome, and typically developing girls and boys.
Caption: Detailed cognitive profile for girls with Turner syndrome (TS), boys with Klinefelter syndrome (KS), typically developing girls (TD girls), and typically developing boys (TD boys) using the NEPSY measure of attention and executive function: Auditory Attention, Response Set, Naming, Inhibition, and Switching. Subtests of the Wechsler Preschool and Primary Scale of Intelligence Third Edition (WPPSI-III; for children < 6 years) or Wechsler Intelligence Scale for Children Fourth Edition (WISC-IV; for children 6 years and older): Digit Span, Letter-Number Sequencing, Coding, and Symbol Search.

Figure 3.
Title: Study design and the measured effects of sex chromosomes variation on ADHD phenotype and executive function.
Caption: The hypothesized effect size of sex chromosomes variation. We compared girls with Turner syndrome and boys with Klinefelter syndrome to their sex-matched typically developing controls. Comparing boys with Klinefelter syndrome (XXY) vs. typically developing boys (XY), theoretically measuring the effect of an X chromosome addition. Comparing girls with Turner syndrome (45, X0) vs. typically developing girls (XX), theoretically measuring effect of missing an X chromosome. Effect size (**rank-biserial correlation, r**): X axis – Non-parametric effect size (confidence interval); Y axis- Turner syndrome vs. typically developing girls (XX) X(-), Klinefelter syndrome (XXY) vs. typically developing boys (XY) X(+). A vertical line was added at **r** = 0 to mark confidence interval significance (47).

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Keywords: ADHD, Turner syndrome, Klinefelter syndrome, Executive Functions, X-chromosome

Contributors' Statement

Dr. Green conceptualized and designed the study, collected data, carried out the final analyses, wort the manuscript, and revised the manuscript.

Drs. Reiss and Hong conceptualized and designed the study, designed the data collection instruments, and reviewed and revised the manuscript.

Dr. Flash conceptualized and designed the study and contributed to the interpretation of data, drafted the initial manuscript, and revised the manuscript.

Ms. Shankar collected and computerized the data and reviewed and revised the manuscript.

Ms. Bade Shrestha collected data, carried out the initial analyses, drafted the initial manuscript, and reviewed and revised the manuscript.

Dr. Jo conceptualized and designed the study, supervised data analyses, and critically reviewed the manuscript for valuable intellectual content.

Dr. Klabunde designed the data collection instruments, critically supervised the data collection, and reviewed and revised the manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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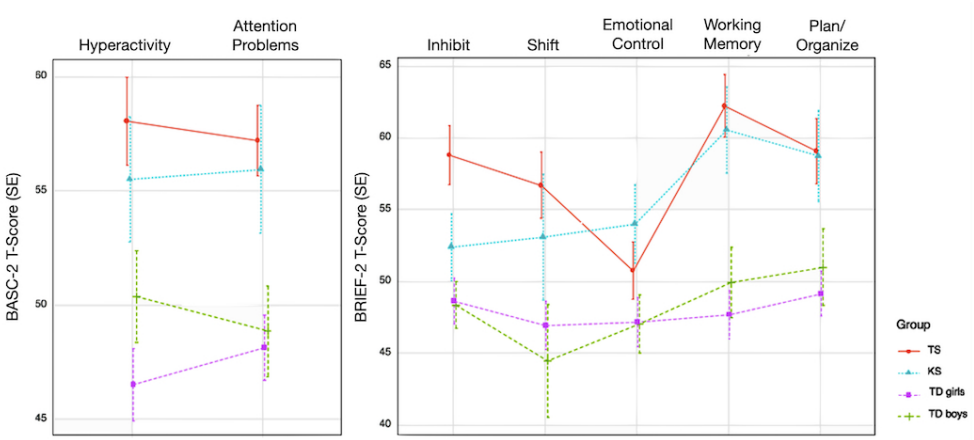
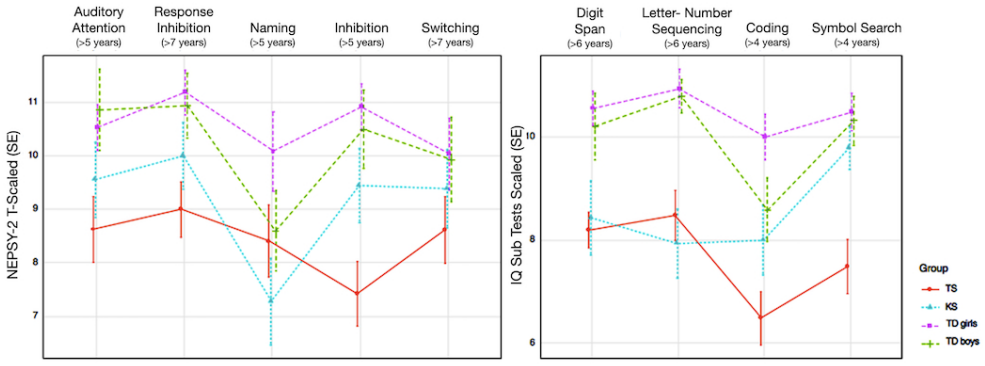


Title: Behavioral symptoms of ADHD and executive functions in girls with Turner syndrome, boys with Klinefelter syndrome, and typically developing girls and boys.

Caption: Behavioral assessment of ADHD symptoms and executive function profile for girls with Turner syndrome (TS), boys with Klinefelter syndrome (KS), typically developing girls (TD girls), and typically developing boys (TD boys). Behavioral symptoms of ADHD were measured using the Behavior Assessment System for Children (BASC-2) Attention Problems and Hyperactivity scales. Executive function profile was measured using the attention and executive function scales/subtests of the Behavior Rating Inventory of Executive Function (BRIEF) scales: Inhibit, Shift, Emotional Control, Working Memory, and Plan/Organize.



Title: Detailed Cognitive profile for girls with Turner syndrome, boys with Klinefelter syndrome, and typically developing girls and boys.

Caption: Detailed cognitive profile for girls with Turner syndrome (TS), boys with Klinefelter syndrome (KS), typically developing girls (TD girls), and typically developing boys (TD boys) using the NEPSY measure of attention and executive function: Auditory Attention, Response Set, Naming, Inhibition, and Switching. Subtests of the Wechsler Preschool and Primary Scale of Intelligence Third Edition (WPPSI-III; for children < 6 years) or Wechsler Intelligence Scale for Children Fourth Edition (WISC-IV; for children 6 years and older): Digit Span, Letter-Number Sequencing, Coding, and Symbol Search

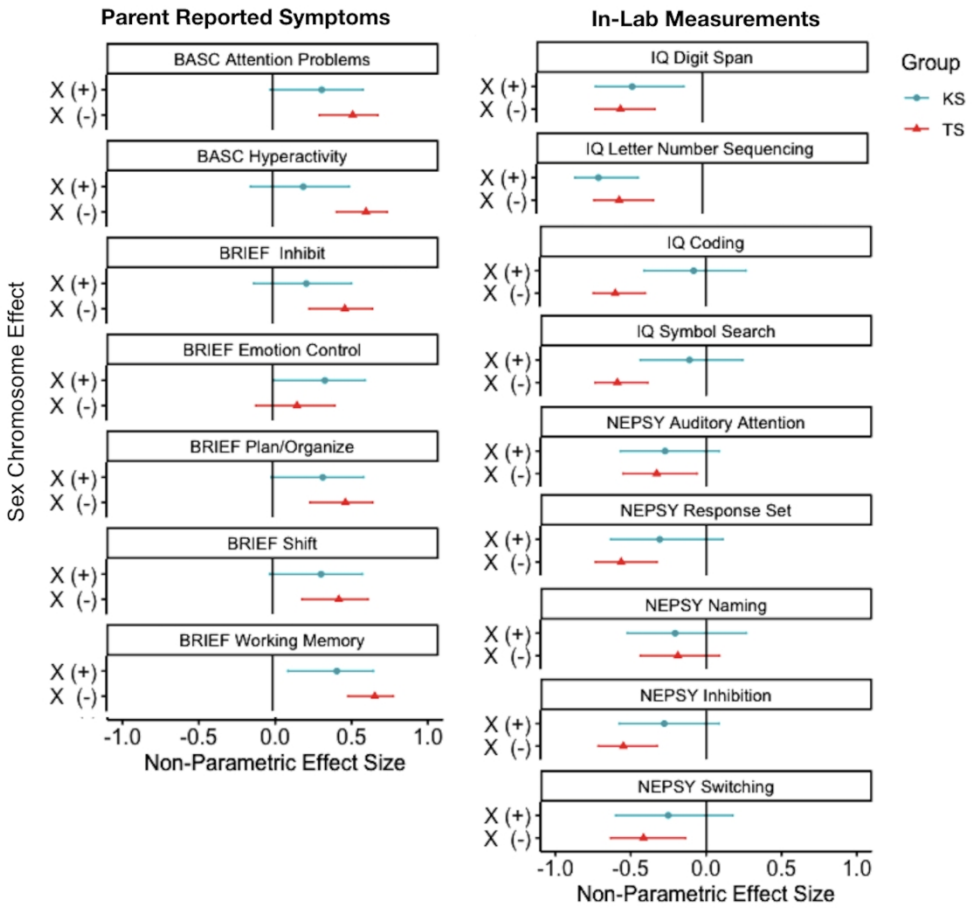


Figure 3.
Title: Study design and the measured effects of sex chromosomes variation on ADHD phenotype and executive function.
Caption: The hypothesized effect size of sex chromosomes variation. We compared girls with Turner syndrome and boys with Klinefelter syndrome to their sex-matched typically developing controls. Comparing boys with Klinefelter syndrome (XXY) vs. typically developing boys (XY), theoretically measuring the effect of an X chromosome addition. Comparing girls with Turner syndrome (45, X0) vs. typically developing girls (XX), theoretically measuring effect of missing an X chromosome. Effect size (rank-biserial correlation, r): X axis – Non-parametric effect size (confidence interval); Y axis- Turner syndrome vs. typically developing girls (XX) X(-), Klinefelter syndrome (XXY) vs. typically developing boys (XY) X(+). A vertical line was added at r = 0 to mark confidence interval significance (47).

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Supplementary materials

Participants

All TS and KS diagnoses were confirmed by karyotype analysis provided in participant medical records. All participants were in good overall medical condition. None of the participants reported any previous or current neurological or psychiatric diagnoses, or were taking psychotropic medications such as stimulants, selective serotonin reuptake inhibitors (SSRIs), or antipsychotic agents at the time of assessment. Thirty-one participants from the TS group were receiving treatment with growth hormone (81.6%), and one participant was receiving estrogen replacement therapy (Table 1). Exclusion criteria for all groups included premature birth (gestational age under 34 weeks), and low birth weight (less than 2000 g.; 4.4 lbs.). All participants were screened by a physician for pubertal status during their visit using Tanner pubertal scale scores (Table 1) (1,2).

Overall Cognitive Measures

Wechsler Preschool and Primary Scale of Intelligence Third Edition (WPPSI-III) (3) was administered to children under the age of 6, and The Wechsler Intelligence Scale for Children Fourth Edition (WISC-IV) (4) was administered to children who were 6 years or older. The following scores were recorded to provide a general assessment of cognitive abilities, which have been previously shown to be affected in KS and TS(5): Full scale Intelligence Quotient (FSIQ; WISC-IV/WPPSI-III heretofore referred to as "IQ"), the Perceptual Reasoning Index (PRI; WISC-IV) or Perceptual Intelligence

Quotient (PIQ; WPPSI-III), the Verbal Comprehension Index (VCI; WISC-IV) or Verbal Intelligence Quotient (VIQ; WPPSI-III), the Working Memory Index (WMI; WISC-IV; an analogous score is not available for the WPPSI-III), and the Processing Speed Index (PSI; WISC-IV) or processing speed Quotient (PSQ; WPPSI-III, children 4 years and older). Generally, WISC-IV and WPPSI-III group means are identical within their age overlap range (6-7.3 years), and FSIQ scores of both test versions are highly correlated, with only the Coding subtest of the PSI/PSQ exhibiting a slight mean difference between tests(6).

Behavioral measures of attention deficit hyperactivity disorder (ADHD)

The Behavior Assessment System for Children-Second Edition Parent Rating Scale (BASC-2) (7) was used to evaluate symptoms of ADHD, including attention problems and hyperactivity. Scales used in the analyses were similar across the two forms: preschool (2-5 years) and child (6-11 years).

Executive Functions (EF) - behavioral, neuropsychological and cognitive assessments

The Behavior Rating Inventory of Executive Function (BRIEF) (8), was used as an assessment of ADHD-related behavioral EF at home, including the following scales: Inhibitory Control, Shifting Attention Control, Emotional Control, Working Memory, and Plan/Organize. Again, only scales that were similar across the two forms were utilized: preschool (2-5) and regular (5-18). All variables were age-normed and scaled.

The NEuroPSYchological test, version 2 (NESPY-2) (9) was used for assessment of neuropsychological domains of EF. Test administrators followed standard procedures as outlined in the published product manuals, and were

supervised by a licensed clinical psychologist, who maintained testing administration and scoring fidelity. All cognitive and neuropsychological variables were age-normed and scaled. Only subtests within the attention and EF domains were of interest in this investigation. The Auditory Attention and Response Set included scores for Auditory Attention (a measure of sustained attention, administered from 5 years of age) and Response Inhibition (a measure of the ability to inhibit an inappropriate response, administered from 7 years of age). The Inhibition Set included scores for Naming (a measure of processing speed, administered from 5 years of age), Inhibition (a measure of inhibitory skills, administered from 5 years of age), and Switching (a measure of the ability to shift cognitive sets and cognitive flexibility, administered from 7 years of age).

Given that participants under the age of 7 could not be tested on several measures from the NEPSY-2, we also analyzed subtests of the WPPSI-III/WISC-IV as additional proxies of EF, specifically focused on working memory and processing speed: Digit Span (IQ-Digit Span; a measure of auditory working memory from the WMI, administered from 6 years of age), Letter-Number Sequencing (IQ-Letter-Number Sequencing; a measure of auditory working memory and sequencing from the WMI of the WISC-IV, administered from 6 years of age), Coding (IQ-Coding; a measure of visual processing speed from the PSI/PSQ, administered from 4 years of age), and Symbol Search (IQ-Symbol Search; a measure of visual processing speed from the PSI of the WISC-IV, administered from 6 years of age).

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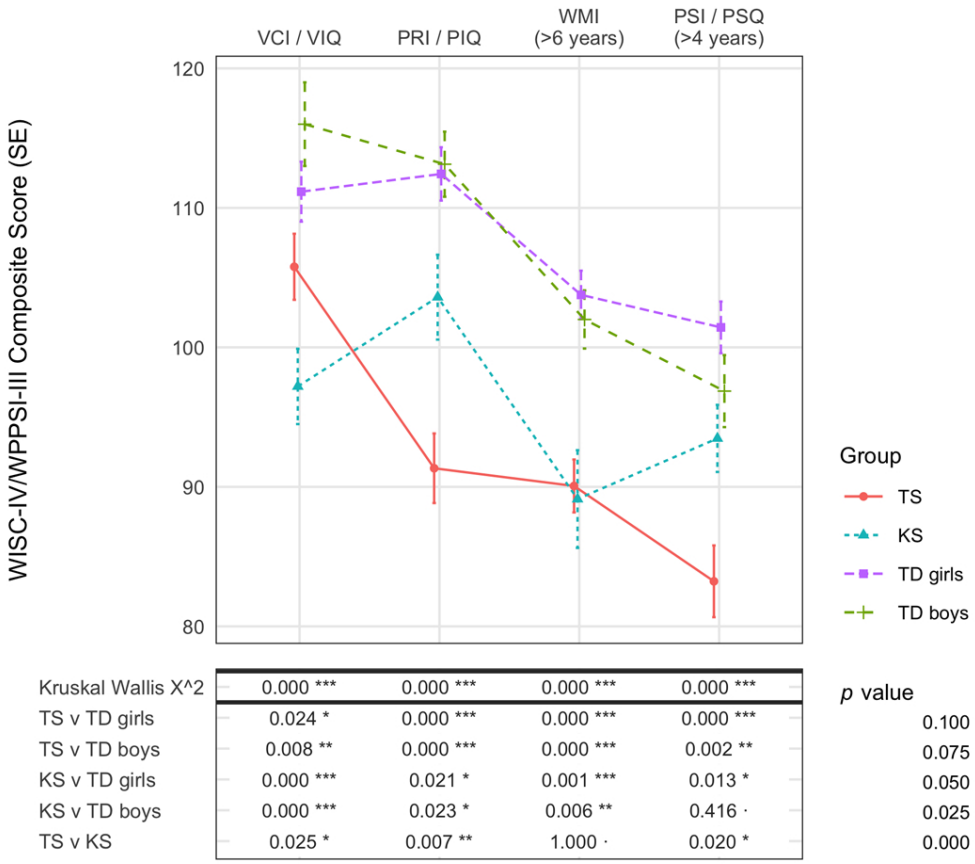

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