Phonemic and Semantic Verbal Fluency in Sex Chromosome Aneuploidy: Contrasting the Effects of Supernumerary X *versus* Y Chromosomes on Performance

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Abstract

Objectives: Past research suggests that youth with sex chromosome aneuploidies (SCAs) present with verbal fluency deficits. However, most studies have focused on sex chromosome trisomies. Far less is known about sex chromosome tetrasomies and pentasomies. Thus, the current research sought to characterize verbal fluency performance among youth with sex chromosome trisomies, tetrasomies, and pentasomies by contrasting how performance varies as a function of extra X number and X *versus* Y status. **Methods:** Participants included 79 youth with SCAs and 42 typically developing controls matched on age, maternal education, and racial/ethnic background. Participants completed the phonemic and semantic conditions of a verbal fluency task and an abbreviated intelligence test. **Results:** Both supernumerary X and Y chromosomes were associated with verbal fluency deficits relative to controls. These impairments increased as a function of the number of extra X chromosomes, and the pattern of impairments on phonemic and semantic fluency differed for those with a supernumerary X *versus* Y chromosome. Whereas one supernumerary Y chromosome was associated with similar performance across fluency conditions, one supernumerary X chromosome was associated with relatively stronger semantic than phonemic fluency skills. **Conclusions:** Verbal fluency skills in youth with supernumerary X and Y chromosomes are impaired relative to controls. However, the degree of impairment varies across groups and task condition. Further research into the cognitive underpinnings of verbal fluency in youth with SCAs may provide insights into their verbal fluency deficits and help guide future treatments. (*JINS*, 2018, *24*, 917–927.)

Keywords: Verbal fluency, Cognition, X chromosome, Y chromosome, Klinefelter syndrome, Trisomy X syndrome

INTRODUCTION

Sex chromosome aneuploidies (SCAs), genetic disorders characterized by an abnormal number of X and/or Y chromosomes, occur collectively at a rate of approximately 1/400 births (Nielsen & Wohlert, 1991). While several SCA variants exist, Klinefelter (47,XXY), Trisomy X (47,XXX), and 47,XYY syndromes are the most common. Prior research has demonstrated that individuals with SCAs are at increased risk for experiencing impairments on verbal fluency tasks (see discussion below). These tasks, which are among the most frequently administered in neuropsychology (Ardila, Ostrosky-Solis, & Bernal, 2006), require individuals to rapidly state words within a specific time frame that either fit into a semantic category (semantic fluency) or start with a particular letter (phonemic fluency) while simultaneously following a set of rules. Successful completion of these tasks requires that individuals have a well-developed, interconnected lexicon and use effective strategies for identifying as many novel words as possible while not repeating previously stated words. Thus, these tasks tap into multiple cognitive domains (Unsworth, Spillers, & Brewer, 2010;

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Henry, Messer, & Nash, 2015) and may provide insight into impaired cognitive processes associated with different SCAs.

One cognitive domain that is relevant for verbal fluency tasks (Whiteside et al., 2016) and is an area of challenge for individuals with SCAs (for review, see Leggett, Jacobs, Nation, Scerif, & Bishop, 2010) is language. Other cognitive domains of interest include episodic memory, processing speed, and different executive functions (e.g., working memory, initiation; Strauss, Sherman, & Spreen, 2006). This latter set of skills, executive functions, are also reported to be impaired youth with SCAs (Lee et al., 2011, 2015; Ross, Zeger, Kushner, Zinn, & Roeltgen, 2009). As verbal fluency has been found to be associated with important functional outcomes in other clinical populations (activities of daily living: Cahn-Weiner, Boyle, & Malloy, 2002; social abilities: Bowie et al., 2008; and academics: Daneman, 1991; Nathan & Abernathy, 2012), further research into the nature of fluency impairments in different SCAs may shed light on cognitive skills that are related to real-world outcomes in these groups.

Few studies have characterized verbal fluency performance in youth with supernumerary SCAs. However, limited research indicates that the presence of a single supernumerary X chromosome is adversely related to performance on phonemic and semantic fluency in females with 47,XXX (Bender, Linden, & Harmon, 2001; Bender, Linden, & Robinson, 1989). Similarly, the addition of an X chromosome in males with 47,XXY has been found to be associated with poorer verbal fluency performance in some studies (phonemic: Bender et al., 1989; Ross et al., 2009; semantic: Bender et al., 2001). Still, inconsistency in the 47,XXY literature exists, as some studies have failed to find differences between males with 47,XXY and controls (semantic: Ross et al., 2009). Research on verbal fluency in males with a supernumerary Y chromosome is scarce. The one published study reported impaired phonemic but not semantic fluency performance in males with 47,XYY relative to typical controls (Ross et al., 2009). However, effect sizes for both conditions were large suggesting more general verbal fluency impairments.

Despite preliminary evidence that individuals with SCAs present with impaired verbal fluency, most studies have been limited to youth with SCA trisomies. No studies have examined verbal fluency across the full spectrum of supernumerary SCAs, including youth with tri-, tetra-, and pentasomies. Moreover, existing studies have not consistently contrasted how phonemic and semantic fluency performance varies as a function of an additional X or Y chromosome.

Thus, the current research aimed to fill these gaps in the literature by answering the following questions:

1. How does the presence of an extra X chromosome impact fluency performance, and does performance worsen with increasing supernumerary X chromosome number? Consistent with prior research (e.g., Lee et al., 2011), it was hypothesized that the presence of an extra X chromosome would be associated with an overall decrement in verbal fluency performance. Moreover, greater impairments with each additional X chromosome were anticipated, consistent with research on supernumerary X effects on overall

cognitive (Polani, 1977) and language (Lee et al., 2012) functioning.

2. How does the presence of an extra Y chromosome impact verbal fluency performance? Consistent with prior research (Ross et al., 2009), we anticipated that the presence of an extra Y chromosome would be associated with a decrement in verbal fluency performance.

3a. Are there differences in the effects of an extra X *versus* Y on verbal fluency performance among males; 3b. Does the presence of both an extra X and an extra Y chromosome (48, XXYY) result in greater impairments in verbal fluency than the presence of an extra X (47,XXY) or extra Y (47,XYY) in isolation? Because prior research has documented somewhat different cognitive profiles for those with supernumerary X *versus* Y SCAs (Lee et al., 2012; Ross et al., 2009), we hypothesized that there would be differential X and Y chromosome effects on verbal fluency. However, given inconsistencies in the literature, it was difficult to predict whether an extra X or Y would impact phonemic *versus* semantic fluency more or less. Lastly, we anticipated that males with both an additional X and additional Y would be more impaired than those with an extra X or Y in isolation, given that prior research indicates that increasing sex chromosome number is associated with increasing impairment (Lee et al., 2012; Polani, 1977).

METHOD

Participants

Seventy-nine youth with SCA participated in this study as part of a larger research program conducted at the National Institute of Mental Health (NIMH) Intramural Research Program. These youth were recruited from parent organizations and by means of the NIMH Web site. Inclusion criteria for the SCA group were as follows. Participants must have (a) had a supernumerary SCA diagnosis confirmed by karyotype, and (b) been free of a comorbid neurological disorder or acquired head injury (e.g., TBI, hydrocephalus) that would impact gross brain development.

In this study, SCA groups were divided based on the number of extra X or Y chromosomes rather than by karyotype. As such, the +1X group included those with 47, XXX and 47,XXY. Because of the rarity of tetra- and pentasomy SCAs, youth with 2 and 3 additional X chromosomes were combined into one group, which we refer to as the +2/3X group. This group consisted of individuals with 48,XXXX, 48, XXXY, and 49,XXXXY. The +1Y group included males with 47,XYY, and +1X, +1Y group included males with 48, XXYY. See Table 1 for demographic information.

Before grouping males and females with supernumerary X chromosomes together for analyses, we compared the performance of the 47,XXY ($n = 29$) and 47,XXX ($n = 27$) groups on verbal fluency measures. Sex differences were not found on phonemic or semantic fluency (Phonemic: 47,XXY M = -1.09 ± .68; 47,XXX M = -.97 ± 1.05; [$t < 1$; $p > .6$]; Semantic: 47,XXY M = -.61 ± 1.15; 47,XXX

Table 1. Demographic information, IQ, and verbal fluency scores by study group and karyotype

Group/karyotype	N	Age M (SD)	Age Range	IQ M (SD)	IQ Range	FAS M (SD)	FAS Range	ANIM M (SD)	ANIM Range	Sex %Male	Race[a] % WNH[b]	Mat. Ed.[c] % college or more
+0X, +0Y (controls)	42	11.67 (2.90)	6.60 to 17.40	116.33 (13.95)	83 to 142	0.38 (0.99)	-1.74 to 3.00	0.21 (1.04)	-1.98 to 3.00	52	81	48
46, XX	20	11.43 (2.93)	7.10 to 16.30	115.3 (16.11)	83 to 140	0.36 (0.91)	-1.59 to 2.46	0.22 (1.00)	-1.53 to 1.82		80	45
46, XY	22	11.89 (2.92)	6.60 to 17.40	117.27 (11.96)	92 to 142	0.40 (1.08)	-1.74 to 3.00	0.20 (1.09)	-1.98 to 3.00		82	50
+1X Group	46	11.06 (3.08)	7.10 to 20.50	96.85 (14.64)	61 to 129	-1.02 (0.91)	-3.20 to 1.08	-0.46 (1.07)	-2.56 to 2.23	41	85	72
47, XXX	27	10.80 (2.93)	7.30 to 18.70	93.81 (15.22)	61 to 129	-0.97 (1.05)	-3.20 to 1.08	-0.35 (1.02)	-1.87 to 2.23		82	78
47, XXY	19	11.44 (3.34)	7.10 to 20.50	101.16 (12.97)	81 to 127	-1.09 (0.68)	-2.26 to 0.18	-0.61 (1.15)	-2.56 to 2.11		90	63
+2/3X Group	12	12.02 (4.38)	7.10 to 17.40	67.00 (10.22)	52 to 81	-1.91 (0.83)	-3.03 to 0.14	-2.13 (0.98)	-3.45 to -0.80	92	75	58
48, XXXX	1	17.33 (–)	–	58.00 (–)	–	-2.61 (–)	–	-3.07 (–)	–		100	100
48, XXXY	4	11.31 (4.44)	7.10 to 17.40	74.50 (6.95)	68 to 81	-1.34 (1.08)	-2.46 to 0.14	-1.22 (0.48)	-1.87 to -0.80		100	50
49, XXXXY[d]	7	11.67 (4.49)	7.70 to 17.20	63.50 (10.19)	52 to 77	-2.13 (0.58)	-3.03 to -1.45	-2.52 (0.876)	-3.45 to -1.17		57	57
+1Y Group/ 47, XYY	11	12.58 (2.87)	7.98 to 16.80	99.27 (12.37)	74 to 114	-0.85 (0.89)	-2.67 to 0.32	-1.22 (0.89)	-3.07 to -0.21	100	100	46
+1X, +1Y Group/ 48,XXYY	10	13.36 (4.52)	7.30 to 22.60	86.5 (16.89)	68 to 121	-1.20 (1.11)	-2.53 to 0.72	-0.40 (0.97)	-1.48 to 1.92	100	100	60

[a] The ethnic/racial composition of the SCA group was as follows: white, non-Hispanic (87.3%), black (1.3%), white, Hispanic (5.1%), Asian (2.5%), and other (3.8%); The ethnic and racial composition for the control group was as follows: white, non-Hispanic (81%), black (7.1%), white, Hispanic (7.1%), Asian (2.4%), and other (2.4%).
[b] White, non-Hispanic.
[c] Maternal Education: percent with college education or higher.
[d] IQ data were missing for one participant with 49, XXXXY.

M = -.35 ± 1.02; [$t < 1$; $p > .4$]). This group comparison could not be made for those with +2/3X, as there was only one female in this group (who had 48,XXXX). Thus, for parsimony in analyses, males and females with supernumerary X chromosomes were combined into groups.

Participants in the SCA sample included those with prenatal and postnatal diagnoses: +1X (70% prenatal, 30% postnatal), +2/3X (100% postnatal), +1Y (46% prenatal, 54% postnatal), and +1X, +1Y (100% postnatal). Given the subtle physical and cognitive phenotype associated with sex chromosome trisomies, youth with these disorders can go undiagnosed (Bojesen, Juul, & Gravholt, 2003). Consequently, youth with postnatal diagnoses may present with more severe impairments than those with prenatal diagnoses, as these impairments may have led to their eventual diagnosis. To examine this possibility, analyses that included youth with +1X or +1Y were re-run with just the prenatal subsample to ensure consistency in findings. Results were largely the same. Thus, study findings are from analyses of youth with both pre- and postnatal diagnoses.

Because the youth with tetra- and pentasomies in our sample all had postnatal diagnoses, we could not complete analogous analyses with a prenatal sample only. However, we were less concerned about ascertainment bias in these groups, particularly in those with +2/3X group, as these SCA variants tend to be associated with greater physical dysmorphology and cognitive impairments. Thus, they are less likely to go undetected by medical providers.

A control group of 42 typically developing (TD) males and females who were part of other studies at the National Institutes of Health (NIH) and recruited with the help of the NIH Healthy Volunteer office were also included. This group, referred to as the +0X, +0Y group, was matched to the SCA group on age, racial/ethnic background, and maternal education. Inclusion criteria for this group included being free of any psychiatric, neurological, and/or learning disorders.

Information regarding age, sex ratio, IQ, maternal education, and racial composition of the groups is summarized in Table 1. As desired, groups did not differ on age ($p > .25$), maternal education (% ≥ college education; $p > .18$), or racial composition (%White, non-Hispanic; $p > .26$). Groups did, however, differ on IQ ($F[4,119] = 32.15$; $p < .001$), such that TD controls had higher IQ scores than all of the SCA groups (all $ps < .001$), and the +2/3X group had lower IQ scores than all other groups ($ps < .05$). These findings were expected, as reductions in overall intellectual functioning are a characteristic of SCAs (Polani, 1977). Lastly, groups differed on sex ratio ($p < .001$) as expected, as karyotypes involving an extra Y occur only in males.

Procedures

The NIH Combined Neuroscience Institutional Review Board approved this study. Participants over the age of majority and parents of minors signed consent forms. Participants under the age of majority also provided assent. Study procedures, including the completion of verbal fluency and an abbreviated intellectual assessment, were completed at the NIH Clinical Center.

Measures

Verbal fluency task

Participants completed both phonemic and semantic fluency conditions. For phonemic, participants stated as many words as they could that started with a F, A, or S during three, 60-s intervals. For semantic, participants stated as many animals as they could during a 60-s interval. Across both conditions, participants were told to avoid repeating words. In the phonemic condition, participants were also instructed to avoid using proper nouns (e.g., names, brands, etc.) and providing minor variations of the same word (e.g., fall, fell). To compare phonemic and semantic fluency performance using the same scale, age-standardized Z-scores for these conditions were generated using norms from Gaddes and Crockett (1975), Halperin, Healey, Zeitchik, Ludman, and Weinstein (1989), and Tombaugh, Kozak, and Rees (1999)[1]. These Z-scores were the primary outcome variables used in the current investigation.

Intelligence (IQ) test

IQ was estimated using the four-subtest Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999) for all but one participant who completed the Wechsler Preschool and Primary Scale of Intelligence—Third edition (Wechsler, 2002).

Statistical Plan

Question 1 focused on whether the presence of an extra X chromosome impacts verbal fluency performance and whether increasing supernumerary X chromosome number relates to greater impairments. For this question, we included all participants *without* an extra Y chromosome and completed a 3 × 2 mixed model analysis of variance (ANOVA), with one between-subjects factor (Extra X number: +0X, +1X, +2/3X) and one within-subjects factor (fluency condition: phonemic, semantic). The second question examined extra Y effects on verbal fluency and included only males from the following groups: +0X, +0Y (TD males: XY) and +1Y (47,XYY). A 2 × 2 mixed model ANOVA was completed with one between-subjects factor (Extra Y number: +0Y, +1Y) and one within-subjects factor (fluency condition). The third question contrasted the effects of a supernumerary X *versus* Y chromosome on fluency in males. To evaluate this, a 2 × 2 × 2 mixed model ANOVA, with two between-subjects factors (presence of an extra X; presence of an extra Y) and one within-subjects factor (fluency condition) was used. This analysis included males with

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[1] Note that means were interpolated based on available age norms for ages 13, 14, and 15.

+ 0X, + 0Y (XY), +1X (47,XXY), +1Y (47,XYY), and +1X, +1Y (48,XXYY).

For primary analyses that examined the effects of an extra X, extra Y, fluency condition, or interaction (of any kind), a false discovery rate (FDR; Benjamini & Hochberg, 1995) experiment-wise adjustment for multiple tests was completed. For the 13 effects that were being examined, the FDR adjusted p-value was .023. Effects that survived FDR correction are noted in a summary table which is described in the Results section. For all aforementioned analyses, in the event of a significant interaction (e.g., group*condition), tests of simple effects that compared performance between pairs of groups and within a group (on condition) were completed with Bonferroni adjustment for multiple comparisons.

Because it was difficult to evaluate normality in the study's small groups (+2/3X group, n = 12; 47,XYY group, n = 11), analyses involving these groups were re-run using one of the following non-parametric tests: Kruskal-Wallis (to evaluate differences among groups when more than two groups were compared); Mann-Whitney U (to evaluate differences between pairs of groups); and Wilcoxon Signed-Rank test (to evaluate within group differences between fluency conditions). Non-parametric results for the questions involving these groups were consistent with results from parametric tests. Because of this convergence in findings and the desire to examine group by verbal fluency condition interactions, only parametric test (mixed model ANOVA) results are reported in the manuscript.

Lastly, before completion of primary analyses, we examined data for outliers (i.e., scores > 3 standard deviations from the sample mean). Our analyses revealed one outlier for phonemic fluency (Z = 3.28) and one for semantic fluency (Z = 4.39). Both were from the TD group. To examine the possible influence of these outliers on study findings, we conducted all primary analyses with and without them included. Results were largely the same. Rather than include or exclude outliers, we elected to take an intermediate approach. Consistent with methods suggested by others (e.g., Ghosh & Vogt, 2012; Tabachnick & Fidell, 2007), we assigned less extreme Z scores to these outliers (Z scores of 3). Because this approach permitted including these individuals while making their scores less influential, primary study results include these two outliers with their adjusted scores.

RESULTS

Phonemic and semantic fluency scores for all groups are summarized in Table 1. Table 2 summarizes findings for the three study questions and displays which study findings survived experiment-wise correction for multiple tests using a FDR adjustment.

In the sections that follow, we outline results by question.

Q1. How does the presence of an extra X chromosome impact fluency performance, and does performance worsen with increasing supernumerary X chromosome number?

Groups: +0X (XX, XY), +1X (47,XXX, 47,XXY), +2/3X (48,XXXX, 48,XXXY, 49,XXXXY).

Results: The 3 × 2 mixed model ANOVA revealed a main effect of extra X ($F[2,97]$ = 43.17; p < .001; η^2_p = .47), such that having additional supernumerary X's was associated with poorer performance overall. However, the main effect of extra X was qualified by a significant extra X*fluency condition interaction ($F[2,97]$ = 5.36; p < .01; η^2_p = .10). Between-group tests of simple effects revealed that all groups differed from one another on phonemic and semantic fluency (+0X > +1X > +2/3; ps < .008; Bonferroni adjustment for six tests). Tests of within-group simple effects revealed that the interaction was driven by the fact that the profile of scores on phonemic and semantic fluency differed by group. Specifically, phonemic and semantic fluency scores were similar within the +0X and +2/3X groups (ps > .35). However, they differed in the +1X such that semantic fluency was less impaired than phonemic fluency (p < .016; Bonferroni adjustment for 3 tests). See Figure 1.

Q2. How does the presence of an extra Y chromosome impact fluency performance?

Groups: +0X, +0Y (XY), +1Y (47,XYY).

Results: A 2 × 2 mixed model ANOVA revealed a main effect of extra Y, such that males with an extra Y chromosome performed worse than controls overall—that is, across conditions ($F[1,31]$ = 18.96; p < .001; η^2_p = .38). However, no extraY*fluency condition interaction was found (p > .6) nor was there a main effect of fluency condition (i.e., there was no evidence of differences in phonemic and semantic fluency scores across the groups; p > .19). Thus, tests of simple effects to evaluate within and between group differences were not completed (see Figure 2).

Q3a. Are there differences in the effects of an extra X *versus* Y on fluency among males?

Q3b. Does the presence of both an extra X and an extra Y chromosome (48,XXYY) result in greater impairments in verbal fluency than the presence of an extra X (47,XXY) or extra Y (47,XYY) in isolation?

Groups: +0X, +0Y males (XY), +1X males (47,XXY), +1Y males (47,XYY), +1X, +1Y males (48,XXYY).

Results: To evaluate questions 3a and 3b, a single 2 × 2 × 2 mixed model ANOVA was completed; however, results are organized below by study question.

Q3a. Consistent with analyses from Q1 in which an extra X was evaluated in isolation (in youth with a somewhat different set of karyotypes), the 2 × 2 × 2 mixed model ANOVA revealed a main effect of extra X ($F[1,58]$ = 4.31; p < .05; η^2_p = .07), such that the addition of an extra X was associated with an overall decrement in verbal fluency performance. Again, this main effect was qualified by an extra X*fluency condition interaction ($F[1,58]$ = 9.18; p < .01; η^2_p = .14). Between group tests of simple effects revealed that when compared to those *without* an extra X (i.e., typical males, XY, and males with 47,XYY), those *with* an extra X (i.e., males with 47, XXY and 48, XXYY) performed less well on phonemic fluency (p < .025; Bonferroni adjustment for 2 comparisons), but similarly on semantic fluency

Table 2. Summary of Main Study Findings

Question	Groups	Between- subjects factors			Within-subjects factor	Between*within interactions			Tests of simple effects
		ME X	ME Y	X*Y	ME Fluency	X* Fluency	Y* Fluency	X*Y* Fluency	
1. X Effect	+0X[a] +1X[b] +2/3X[c]	Yes†	n/a	n/a	No	Yes†	n/a	n/a	Extra X*Fluency Interaction: Between: P: +0X>+1X>+2/3X S: +0X~+1X; +0X & 1X >+2/3X Within: +0X: P~S; +1X: P<S; +2/3X: P~S
2. Y Effect	+0Y[d] +1Y[e]	n/a	Yes†	n/a	No	n/a	No	n/a	N/A (no interaction)
3. X vs Y Effects in males	+0X+0Y[f] +1X[g] +1Y[h] +1X+1Y[i]	Yes	Yes†	Yes†	No	Yes†	No	No	Extra X*Extra Y Interaction Fluency: +0X>+1X~+1Y~+1X+1Y Extra X*Fluency Interaction Between: P: +0X>+1X S: +0X~+1X Within: +0X: P~S; +1X: P<S

Note. Karyotypes included in different groups were as follows: [a]+0X (46,XX, 46,XY); [b]+1X (47,XXY, 47,XXX); [c]+2/3X (48,XXXX, 48,XXXY, 49,XXXXY); [d]+0Y (46,XY); [e]+1Y (47,XYY); [f]+0X+0Y (46,XY); [g]+1X (47,XXY); [h]+1Y(47,XYY); [i]+1X+1Y (48,XXYY)
†Survives FDR correction for multiple tests (13 primary tests; adjusted p-value=.02); Tests of simple effects are Bonferroni corrected. (See text for details).
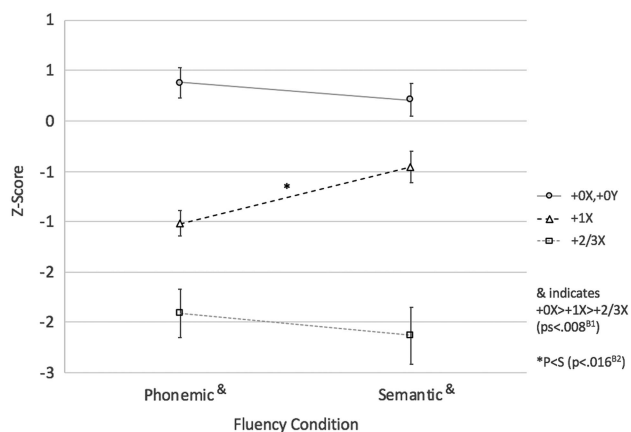ME = main effect; P = Phonemic; S = Semantic.

Fig. 1. Effect of extra X on phonemic and semantic fluency performance. [B1]$p < .008$ (Bonferroni correction for six tests). [B2]$p < .016$ (Bonferroni correction for three tests).
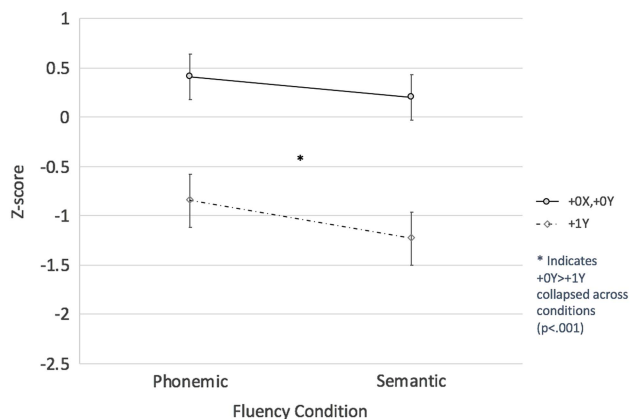

Fig. 2. Extra Y Effect on Phonemic and Semantic Fluency Performance

Fig. 3. Extra X *versus* Y Effects on Fluency Performance among Males.

Note: $+0X, +0Y >$ all groups on verbal fluency (collapsed across conditions); $p < .008$ (Bonferroni correction for 6 tests)

outperformed all three SCA groups (ps $< .008$; Bonferroni adjustment for six comparisons) on verbal fluency overall (collapsed across conditions). However, contrary to expectations, overall verbal fluency performance of males with $+1X, +1Y$ (48, XXYY) was similar to that of males with $+1X$ (47, XXY) and males with $+1Y$ (47, XYY) who in turn did not differ from one another. Finally, there was no extra X*extra Y*fluency condition interaction ($p > .4$) (see Figure 3).

DISCUSSION

The current study sought to characterize verbal fluency performance in youth with SCA tri-, tetra-, and pentasomies. We tested whether the presence of one or more supernumerary sex chromosomes resulted in impaired performance on both phonemic and semantic fluency and whether performance varied as a function of supernumerary X *versus* Y chromosome status. In addition, we evaluated how verbal fluency performance was impacted by increasing supernumerary X chromosome number and contrasted performance of males with $+1X, +1Y$ (48,XXYY) to those with either an extra X (47,XXY) or an extra Y (47,XYY) in isolation.

First, with regard to supernumerary X chromosome influences, our findings indicate that while an extra X exerts a downward influence on verbal fluency overall, semantic fluency appears less impaired than phonemic fluency in youth with $+1X$. These findings are largely consistent with prior research examining verbal fluency in 47,XXY and 47,XXX considered separately (e.g., Bender et al., 1989; Bender et al., 2001; Ross et al., 2009). However, as mentioned, some studies have failed to detect group differences in verbal fluency for males with 47,XXY relative to TD controls (Semantic: Ross et al., 2009). In considering these inconsistencies, it should be noted that effect sizes for group differences in aforementioned studies were medium to large, suggesting that group differences were present but were not statistically significant due to smaller samples.

($p > .3$). Within-group tests of simple effects revealed that phonemic fluency was more impaired than semantic fluency in youth with an extra X ($p < .025$; Bonferroni adjustment for 2 comparisons). This was not the case for those without an extra X ($p > .2$).

Consistent with analyses for Q2, when the effects of an extra Y were evaluated in isolation (in youth with a somewhat different set of karyotypes), the $2 \times 2 \times 2$ mixed model ANOVA also revealed a main effect of extra Y ($F[1,58] = 8.47$; $p < .01$; $\eta^2_p = .13$), such that an extra Y was associated with an overall decrement in performance on verbal fluency. Stated another way, lower scores (collapsed across fluency conditions) were found for those with an extra Y (i.e., males with 47, XYY and 48, XXYY) compared to those without (i.e., typical males, XY, and males with 47, XXY). Again, there was no Y*fluency interaction ($p > .8$) nor was there a main effect of fluency ($p > .2$).

Q3b. The results of the $2 \times 2 \times 2$ mixed model ANOVA also revealed an extra X*extra Y interaction ($F[1,58] = 9.85$; $p < .01$; $\eta^2_p = .15$). Between-group tests of simple effects revealed that those with $+0X, +0Y$ (i.e., typical males)
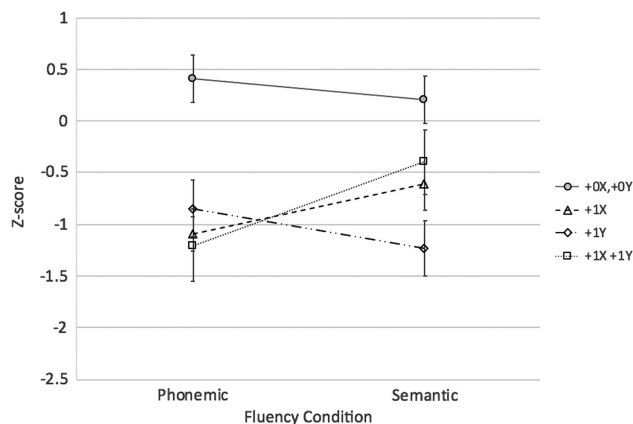
Consistent with our prior work on language functioning (Lee et al., 2012), we found evidence that increasing supernumerary X chromosome number was associated with increased verbal fluency impairments. Specifically, TD youth outperformed youth with X chromosome trisomies, who in turn outperformed youth with X chromosome tetra- and pentasomies on both phonemic and semantic fluency.

Unlike X chromosome effects, only a main effect of extra Y was revealed. There was no Y*fluency condition interaction (nor was there a main effect of verbal fluency condition), suggesting that an extra Y impacted phonemic and semantic fluency similarly. This finding is generally in line with past research (e.g., Ross et al., 2009) in which medium to large effect sizes on semantic and phonemic fluency have been found relative to controls.

Lastly, to contrast X and Y chromosome effects in males only using a 2 (Extra X: 0 or 1) by 2 (Extra Y: 0 or 1) design, performance of the +0X, +0Y (XY), +1X (47,XXY), +1Y (47,XYY), and +1X, +1Y (48, XXYY) groups was contrasted. Findings replicated those reported above that examined these effects individually in a different subset of participants with SCAs. Specifically, in this subset of males, an extra Y was associated with an overall decrement in performance while an extra X was associated lesser semantic than phonemic fluency impairments. While not surprising, these results were not inevitable given differences in the analytic approach and subset of SCA participants included to answer this question. However, they provide further support for possibly differential effects of supernumerary X and Y chromosomes on verbal fluency performance.

Given the limited existing literature comparing subtleties in the neuropsychological phenotype among males with an extra X versus Y, it is difficult to make conclusions about what aspects of the cognitive phenotype for these different disorders are driving these findings. One possibility is that differences in the nature of language impairments experienced by these groups are impacting performance. For example, those with an additional X may have a narrower language impairment that influences phonological more than semantic processing, similar to individuals with dyslexia (Cavalli, Duncan, Elbro, Ahmadi, & Cole, 2017) or isolated speech sound disorder without concomitant global language learning difficulties (Raitano, Pennington, Tunick, Boada, & Shriberg, 2004).

Consistent with this possibility is research that has demonstrated phonemic but not semantic fluency impairments in individuals with dyslexia (e.g., Smith-Spark, Henry, Messer, & Ziecik, 2017). In contrast, males with an extra Y may have a more global language impairment, impacting phonological and semantic processing more broadly, similar to youth with developmental language disorders (Bishop & Snowling, 2004; Claessen, Leitão, Kane, & Williams, 2013) and youth with reading impairments impacting both phonological decoding and comprehension (Snowling & Hulme, 2012).

Alternatively, differences in executive control between the groups could be a contributing factor, as some research has suggested that phonemic fluency task performance may be particularly impacted by executive demands (Luo, Luk, and Bialystok, 2010). However, research on this topic is inconsistent, as other studies (Shao, Janse, Visser, & Meyer, 2014) have reported no differences in the relations between executive abilities and phonemic and semantic fluency performance. Given that there is some research to suggest that males with an extra Y have higher rates of attention-deficit/hyperactivity disorder (ADHD) than males with an extra X (Tartaglia, Ayari, Hutaff-Lee, & Boada, 2012), greater executive dysfunction impairments may characterize 47, XYY and thus could contribute to overall decrements in fluency performance that did not vary by condition. Future studies should begin to disentangle the linguistic and executive underpinnings of verbal fluency in youth with supernumerary X versus Y SCAs. More nuanced descriptions of the cognitive underpinnings of performance on complex tasks such as verbal fluency in youth with SCAs may inform the implementation of more nuanced interventions for this group.

Lastly, we found an extra X*Y interaction, such that males with +1X, +1Y did not differ from males with an isolated extra X or Y when verbal fluency was considered across conditions, that is, the presence of both an extra X and extra Y did not reduce performance in an additive or multiplicative manner. This finding was unexpected, as prior research suggests that increasing sex chromosome number is associated with poorer performance on measures of language and intellectual functioning (Lee et al., 2012; Polani, 1977). It is difficult to interpret this unexpected finding, given that limited research exists contrasting cognitive abilities in youth with different SCA variants. Thus, future research is needed to begin to understand how differing numbers of supernumerary X and Y chromosomes impact cognition and the brain more broadly. This topic is discussed next.

Taken together, this study's results point to both general and specific effects of supernumerary X versus Y chromosomes on verbal fluency. Generally, the presence of an extra X or Y chromosome is associated with poorer verbal fluency performance. Prior studies of brain anatomy in our cohort have identified convergent supernumerary X- and Y-chromosome effects on a large swath of cortical thickness in the frontal lobe as well as proportional cortical thickness of several left-lateralized brain regions central to canonical language networks (Raznahan et al., 2014). The converged effects documented for supernumerary X and Y chromosome dosage on verbal fluency overall echo this anatomical convergence in X/Y effects, suggesting a potential link between patterns of changes in brain and behavior in SCAs.

However, when considering youth with chromosomal trisomies in particular, it appears that an extra X chromosome influences semantic fluency less than phonemic fluency. This pattern was not seen in males with 47,XYY, as only a main effect of extra Y was found; no Y*condition interaction or main effect of fluency was detected. As the literature on neuroanatomical differences associated with supernumerary X and Y chromosomes is limited, it is difficult to make hypotheses about the neural basis of these subtle verbal

fluency differences. Further work is needed to directly assess the degree to which anatomical differences are correlated with cognitive differences in those with supernumerary X and Y SCAs.

This study has several limitations. First, like most recent studies of youth with SCAs (e.g., Tartaglia et al., 2017; van Rijn & Swaab, 2015), some of the participants in our sample were postnatally ascertained, which may have biased results. However, inclusion of this group cannot account for our findings, as results were largely consistent when analyses were re-run with only the prenatal subsample. Another limitation of the current research is the small sample sizes of the tetra- and pentasomy groups. Given the rarity of these conditions, small sample sizes are inevitable for single-site studies. Thus, future research may benefit from combining samples across sites to increase sample size for rarer SCAs. Third, we were not able to examine the contributions of testosterone insufficiency (or supplementation) on performance in males with supernumerary X chromosomes, as testosterone replacement therapy is routinely recommended for adolescent males with these conditions (Wikström & Dunkel, 2011). Lastly, as briefly summarized in the introduction, research suggests that verbal fluency tasks measure multiple domains of cognition, including language and executive abilities (e.g., Henry & Crawford, 2004, 2005; Henry et al., 2015; Ruff, Light, Parker, & Levin, 1997). Thus, it is difficult to make strong conclusions about more nuanced cognitive underpinnings of the verbal fluency impairments faced by youth with different SCAs. Thus, future research should explore the cognitive correlates of verbal fluency tasks in these groups.

Acknowledging these limitations, the current study's findings contribute to the literature on genetic influences on cognition and suggest that genes on the X and Y chromosomes may be important to examine when investigating the genetic underpinnings of verbal fluency impairments, and likely both language and executive function impairments, in other developmental disorders. To truly understand these relationships, future research should combine genetic expression studies, detailed cognitive phenotyping, and neuroimaging. Additionally, murine models of SCAs (e.g., Raznahan et al., 2015) may provide insights into the genetic underpinnings of atypical brain development in these groups.

Lastly, another avenue for future research involves an examination of factors that influence individual differences in verbal fluency performance within SCA groups, as there was significant variability in fluency performance within the different SCA groups. Thus, future research should add to the small literature investigating factors that relate to these individual differences (e.g., Bender, Linden, & Robinson, 1987; van Rijn, Barneveld, Descheemaeker, Giltay, & Swaab, 2018) to identify possible biological and psychosocial factors that could be targets of treatment in future research.

Taken together, findings from our study provide further support that youth with SCAs are at an increased risk for presenting with impairments in verbal fluency and related executive function and language tasks. These challenges are consistent with the comorbid learning and psychiatric conditions that occur at higher rates in youth with SCAs. Specifically, prior research demonstrates that children with SCAs are substantially more likely than TD peers to have ADHD (Tartaglia et al., 2012), dyslexia (Simpson et al., 2014), language disorders (Simpson et al., 2014), and other psychiatric/cognitive impairments (Bruining, Swaab, Kas, & van Engeland, 2009; Rovet, Netley, Bailey, Keenan, & Stewart, 1995). For example, Tartaglia et al. (2012) found that 58% of their sample (47,XXY, 47,XXX, 47,XYY, 48,XXYY) met criteria for ADHD and 74% met criteria for a learning disability.

Still, there is considerable variability within groups for each of these conditions. Consequently, further research is needed to identify neuropsychological and neuroanatomical/physiological predictors of risk for these comorbid conditions in youth with SCAs. As studies of youth without SCAs have shown that verbal fluency is a useful measure to differentiate children with typical development from those with ADHD (Abreu et al., 2013) or dyslexia (Moura. Simões, & Pereira, 2015), this measure may prove to be a clinically useful early indicator of risk for developing these conditions in children with SCAs. Thus, future research on relations between verbal fluency, language, reading, and attentional deficits in youth with SCAs is needed. Such research may permit the early identification of youth with SCAs who are likely to struggle most in these domains and allow for the implementation of targeted and potentially preventative interventions that could improve real world outcomes in individuals with SCAs.

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REFERENCES

Abreu, N., Argollo, N., Oliveira, F., Cardoso, A. L., Bueno, J. L., & Xavier, G. F. (2013). Semantic and phonologic verbal fluency tests for adolescents with ADHD. *Clinical Neuropsychiatry*, *10*(2), 63–71.

Ardila, A., Ostrosky-Solís, F., & Bernal, B. (2006). Cognitive testing toward the future: The example of Semantic Verbal Fluency (ANIMALS). *International Journal of Psychology*, *41*(5), 324–332.

Bender, B. G., Linden, M. G., & Harmon, R. J. (2001). Neuropsychological and functional cognitive skills of 35 unselected adults with sex chromosome abnormalities. *American Journal of Medical Genetics*, *102*(4), 309–313.

Bender, B. G., Linden, M. G., & Robinson, A. (1987). Environment and developmental risk in children with sex chromosome abnormalities. *Journal of the American Academy of Child Adolescent Psychiatry*, *26*(4), 499–503. doi:10.1097/00004583-198707000-00006

Bender, B. G., Linden, M. G., & Robinson, A. (1989). Verbal and spatial processing efficiency in 32 children with sex chromosome abnormalities. *Pediatric Research*, *25*(6), 577–579.

Benjamini, Y., & Hochberg, Y. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, 57, 289–300.

Bishop, D. V., & Snowling, M. J. (2004). Developmental dyslexia and specific language impairment: Same or different?. *Psychological Bulletin*, 130(6), 858.

Bojesen, A., Juul, S., & Gravholt, C. H. (2003). Prenatal and postnatal prevalence of Klinefelter syndrome: A national registry study. *Journal of Clinical Endocrinology & Metabolism*, 88(2), 622–626. doi:10.1210/jc.2002-021491

Bowie, C. R., Leung, W. W., Reichenberg, A., McClure, M. M., Patterson, T. L., Heaton, R. K., & Harvey, P. D. (2008). Predicting schizophrenia patients' real-world behavior with specific neuropsychological and functional capacity measures. *Biological Psychiatry*, 63(5), 505–511.

Bruining, H., Swaab, H., Kas, M., & van Engeland, H. (2009). Psychiatric characteristics in a self-selected sample of boys with Klinefelter syndrome. *Pediatrics*, 123(5), e865–e870.

Cahn-Weiner, D. A., Boyle, P. A., & Malloy, P. F. (2002). Tests of executive function predict instrumental activities of daily living in community-dwelling older individuals. *Applied Neuropsychology*, 9(3), 187–191.

Cavalli, E., Duncan, L. G., Elbro, C., El Ahmadi, A., & Colé, P. (2017). Phonemic–Morphemic dissociation in university students with dyslexia: An index of reading compensation? *Annals of dyslexia*, 67(1), 63–84.

Claessen, M., Leitão, S., Kane, R., & Williams, C. (2013). Phonological processing skills in specific language impairment. *International Journal of Speech-Language Pathology*, 15(5), 471–483.

Daneman, M. (1991). Working memory as a predictor of verbal fluency. *Journal of Psycholinguistic Research*, 20(6), 445–464.

Gaddes, W. H., & Crockett, D. J. (1975). The Spreen-Benton aphasia tests, normative data as a measure of normal language development. *Brain and Language*, 2(3), 257–280.

Ghosh, D., & Vogt, A. (2012). Outliers: An evaluation of methodologies. In *Joint statistical meetings* (pp. 3455–3460). San Diego, CA: American Statistical Association.

Halperin, J. M., Healey, J. M., Zeitchik, E., Ludman, W. L., & Weinstein, L. (1989). Developmental aspects of linguistic and mnestic abilities in normal children. *Journal of Clinical Experimental Neuropsychology*, 11(4), 518–528.

Henry, L. A., Messer, D. J., & Nash, G. (2015). Executive functioning and verbal fluency in children with language difficulties. *Learning and Instruction*, 39, 137–147.

Henry, J., & Crawford, J. (2005). A meta-analytic review of verbal fluency deficits in schizophrenia relative to other neurocognitive deficits. *Cognitive Neuropsychiatry*, 10(1), 1–33.

Henry, J. D., & Crawford, J. R. (2004). A meta-analytic review of verbal fluency performance following focal cortical lesions. *Neuropsychology*, 18(2), 284.

Lee, N. R., Anand, P., Will, E., Adeyemi, E. I., Clasen, L. S., Blumenthal, J. D, … Edgin, J. O. (2015). Everyday executive functions in Down syndrome from early childhood to young adulthood: Evidence for both unique and shared characteristics compared to youth with sex chromosome trisomy (XXX and XXY). *Frontiers in Behavioral Neuroscience*, 9, 264. doi:10.3389/fnbeh.2015.00264

Lee, N. R., Wallace, G. L., Adeyemi, E. I., Lopez, K. C., Blumenthal, J. D., Clasen, L. S., & Giedd, J. N. (2012). Dosage effects of X and Y chromosomes on language and social functioning in children with supernumerary sex chromosome aneuploidies: Implications for idiopathic language impairment and autism spectrum disorders. *Journal of Child Psychology and Psychiatry*, 53(10), 1072–1081.

Lee, N. R., Wallace, G. L., Clasen, L. S., Lenroot, R. K., Blumenthal, J. D., White, S. L., … Giedd, J. N. (2011). Executive function in young males with Klinefelter (XXY) syndrome with and without comorbid attention-deficit/hyperactivity disorder. *Journal of the International Neuropsychological Society*, 17(3), 522–530.

Leggett, V., Jacobs, P., Nation, K., Scerif, G., & Bishop, D. V. (2010). Neurocognitive outcomes of individuals with a sex chromosome trisomy: XXX, XYY, or XXY: A systematic review. *Developmental Medicine & Child Neurology*, 52(2), 119–129.

Luo, L., Luk, G., & Bialystok, E. (2010). Effect of language proficiency and executive control on verbal fluency performance in bilinguals. *Cognition*, 114(1), 29–41.

Moura, O., Simões, M. R., & Pereira, M. (2015). Executive functioning in children with developmental dyslexia. *The Clinical Neuropsychologist*, 28(1), 20–41.

Nathan, A. M., & Abernathy, T. V. (2012). The impact of verbal skills on writing: A comparison of fifth-grade students with learning disabilities and students with typical development. *The Researcher*, 24(2), 96–112.

Nielsen, J., & Wohlert, M. (1991). Sex chromosome abnormalities found among 34,910 newborn children: Results from a 13-year incidence study in Arhus, Denmark. *Birth Defects Original Article Series*, 26(4), 209–223.

Polani, P. E. (1977). Abnormal sex chromosomes, behavior and mental disorder. In J. Tanner (Ed.), *Developments in psychiatric research* (pp. 89–128). London: Hodder and Stoughton.

Raitano, N. A., Pennington, B. F., Tunick, R. A., Boada, R., & Shriberg, L. D. (2004). Pre-literacy skills of subgroups of children with speech sound disorders. *Journal of Child Psychology and Psychiatry*, 45(4), 821–835.

Raznahan, A., Lee, N. R., Greenstein, D., Wallace, G. L., Blumenthal, J. D., Clasen, L. S., & Giedd, J. N. (2014). Globally divergent but locally Convergent X- and Y-Chromosome Influences on cortical development. *Cerebral Cortex*, 26(1), 70–79. doi:10.1093/cercor/bhu174

Raznahan, A., Lue, Y., Probst, F., Greenstein, D., Giedd, J., Wang, C., … Swerdloff, R. (2015). Triangulating the sexually dimorphic brain through high-resolution neuroimaging of murine sex chromosome aneuploidies. *Brain Structure and Function*, 220(6), 3581–3593. doi:10.1007/s00429-014-0875-9

Ross, J. L., Zeger, M. P., Kushner, H., Zinn, A. R., & Roeltgen, D. P. (2009). An extra X or Y chromosome: Contrasting the cognitive and motor phenotypes in childhood in boys with 47, XYY syndrome or 47, XXY Klinefelter syndrome. *Developmental Disabilities Research Reviews*, 15(4), 309–317.

Rovet, J., Netley, C., Bailey, J., Keenan, M., & Stewart, D. (1995). Intelligence and achievement in children with extra X aneuploidy: A longitudinal perspective. *American Journal of Medical Genetics*, 60(5), 356–363.

Ruff, R. M., Light, R. H., Parker, S. B., & Levin, H. S. (1997). The psychological construct of word fluency. *Brain and Language*, 57(3), 394–405.

Shao, Z., Janse, E., Visser, K., & Meyer, A. S. (2014). What do verbal fluency tasks measure? Predictors of verbal fluency performance in older adults. *Frontiers in Psychology*, 5, 772. doi:10.3389/fpsyg.2014.00772

Simpson, N. H., Addis, L., Brandler, W. M., Slonims, V., Clark, A., Watson, J. … Fairfax, B. P. (2014). Increased prevalence of sex chromosome aneuploidies in specific language impairment and dyslexia. *Developmental Medicine Child Neurology*, 56(4), 346–353.

Smith-Spark, J. H., Henry, L. A., Messer, D. J., & Zięcik, A. P. (2017). Verbal and non-verbal fluency in adults with developmental dyslexia: Phonological processing or executive control problems?. *Dyslexia*, *23*(3), 234–250.

Snowling, M. J., & Hulme, C. (2012). Annual research review: The nature and classification of reading disorders–a commentary on proposals for DSM-5. *Journal of Child Psychology and Psychiatry*, *53*(5), 593–607.

Strauss, E., Sherman, E. M., & Spreen, O. (2006). *A compendium of neuropsychological tests: Administration, norms, and commentary*. New York: American Chemical Society.

Tabachnick, B. G., & Fidell, L. S. (2007). *Using multivariate statistics* (5th ed.). New York: Allyn and Bacon.

Tartaglia, N. R., Wilson, R., Miller, J. S., Rafalko, J., Cordeiro, L., Davis, S., … Ross, J. (2017). Autism spectrum disorder in males with sex chromosome aneuploidy: XXY/Klinefelter Syndrome, XYY, and XXYY. *Journal of Developmental and Behavioral Pediatrics*, *38*(3), 197–207. doi:10.1097/DBP.0000000000000429

Tartaglia, N. R., Ayari, N., Hutaff-Lee, C., & Boada, R. (2012). Attention-deficit hyperactivity disorder symptoms in children and adolescents with sex chromosome aneuploidy: XXY, XXX, XYY, and XXYY. *Journal of Developmental and Behavioral Pediatrics*, *33*(4), 309–318.

Tombaugh, T. N., Kozak, J., & Rees, L. (1999). Normative data stratified by age and education for two measures of verbal fluency: FAS and animal naming. *Archives of Clinical Neuropsychology*, *14*(2), 167–177.

Unsworth, N., Spillers, G. J., & Brewer, G. A. (2010). Variation in verbal fluency: A latent variable analysis of clustering, switching, and overall performance. *The Quarterly Journal of Experimental Psychology, 64*(3), 447–466.

van Rijn, S., Barneveld, P., Descheemaeker, M. J., Giltay, J., & Swaab, H. (2018). The effect of early life stress on the cognitive phenotype of children with an extra X chromosome (47,XXY/47, XXX). *Child Neuropsychology*, *24*(2), 277–286. doi:10.1080/09297049.2016.1252320

van Rijn, S., & Swaab, H. (2015). Executive dysfunction and the relation with behavioral problems in children with 47, XXY and 47, XXX. *Genes, Brain, and Behavior*, *14*(2), 200–208.

Wechsler, D. (1999). *Manual for the Wechsler abbreviated intelligence scale*. San Antonio, TX: The Psychological Corporation.

Wechsler, D. (2002). The Wechsler Preschool and Primary Scale of Intelligence – Third Edition. San Antonio, TX: The Psychological Corporation.

Whiteside, D. M., Kealey, T., Semla, M., Luu, H., Rice, L., Basso, M. R., … Roper, B. (2016). Verbal fluency: Language or executive function measure? *Applied Neuropsychology: Adult*, *23*(1), 29–34. doi:10.1080/23279095.2015.1004574

Wikström, A. M., & Dunkel, L. (2011). Klinefelter syndrome. *Best Practice and Research in Clinical Endocrinology and Metabolism*, *25*(2), 239–250. doi:10.1016/j.beem.2010.09.006