Article details

Samango-Sprouse C., Stapleton E., Chea S., Lawson P., Sadeghin T., Cappello C., De Sonneville L. & Van Rijn S. (2019), International investigation of neurocognitive and behavioral phenotype in 47,XXY (Klinefelter syndrome): Predicting individual differences, American Journal of Medical Genetics 176(4): 877-885.
Doi: 10.1002/ajmg.a.38621

Received: 6 June 2017 | Revised: 27 October 2017 | Accepted: 4 January 2018
DOI: 10.1002/ajmg.a.38621

ORIGINAL ARTICLE

WILEY AMERICAN JOURNAL OF medical genetics PART A

International investigation of neurocognitive and behavioral phenotype in 47,XXY (Klinefelter syndrome): Predicting individual differences

Carole Samango-Sprouse[1,2,3] | Emily Stapleton[1] | Selena Chea[1] | Patrick Lawson[1] | Teresa Sadeghin[1] | Chris Cappello[1] | Leo de Sonneville[4] | Sophie van Rijn[4]

[1] The Focus Foundation, Davidsonville, Maryland

[2] George Washington University, Washington, District of Columbia

[3] Florida International University, Miami, Florida

[4] Leiden University, Rapenburg, Leiden, Netherlands

Correspondence
Carole Samango-Sprouse, 820 W. Central Ave. #190, Davidsonville 21035, MD.
Email: cssprouse@email.gwu.edu

Funding information
VENI Grant, Grant number: 016.095.060

47,XXY (KS) occurs in 1:650 male births, though less than 25% are ever identified. We assessed stability of neurocognitive features across diverse populations and quantified factors mediating outcome. Forty-four boys from the Netherlands (NL) and 54 boys from the United States (US) participated. The Wechsler Intelligence Scales assessed intellectual functioning; the ANT program evaluated cognitive function; and the CBCL assessed behavioral functioning. ANOVA was used for group comparisons. Hierarchical regressions assessed variance explained by each independent variable: parental education, timing of diagnosis, testosterone, age, and nationality. Parental education, timing of diagnosis, and hormonal treatment all played an important role in neurocognitive performance. The observed higher IQ and better attention regulation in the US group as compared to the NL group was observed with decreased levels of behavioral problems in the US group. Cognitive measures that were different between the NL and US groups, i.e., attention regulation and IQ scores, were also significantly influenced by external factors including timing of diagnosis, testosterone treatment, and parental education. On the ANT, a cognitive phenotype of 47,XXY was observed, with similar scores on 9 out of the 10 ANT subtests for the NL and US groups. This study lays additional features to the foundation for an algorithm linking external variables to outcome on various neurodevelopmental measures.

KEYWORDS
47,XXY, Klinefelter syndrome, sex chromosome, sex chromosome aneuploidy, XXY

1 | INTRODUCTION

47,XXY (Klinefelter Syndrome) is the most common X and Y chromosomal variation occurring in between 1:500 and 1:1000 live male births (Nielsen & Wohlert, 1991; Perwein, 1984). However, only 10% of 47,XXY cases are diagnosed prenatally, and less than 25% of cases are ever diagnosed (Abramsky & Chapple, 1997; Bojesen, Juul & Gravholt, 2003). The neurodevelopmental profile of boys with 47,XXY is variable but includes language-based learning difficulties, developmental dyspraxia, executive dysfunction, and an increased risk of social cognitive impairments (Boada, Janusz, Hutaff-Lee, & Tartaglia, 2009; Leggett, Jacobs, Nation, Scerif, & Bishop, 2010; Ross et al., 2012; Samango-Sprouse & Rogol, 2002; Simpson et al., 2003).

While consistent deficiencies are observed across multiple studies, there is variability in both function and presentation. For instance, estimates of ADHD in boys with 47,XXY range from 34% to

63% (Lee et al., 2011; Tartaglia, Ayari, Hutaff-Lee, & Boada, 2012). Wide divergence is also observed in the intelligence profile of boys with 47,XXY, with the majority of boys exhibiting intact cognitive profiles and IQs within the normal range (and some in the gifted range), and few with mild intellectual disabilities (Leggett et al., 2010; Simpson et al., 2003). A host of genetic and environmental factors are influential in this phenotypic variability, although the relative contributions of these multiple factors remains unclear. Genetic influences include skewed X inactivation (Iitsuka et al., 2001), parental origin of the additive X chromosome (Bruining et al., 2010), mosaicism (Mohd & Jalaludin, 2016), and a positive family history of learning disabilities (Samango-Sprouse, Stapleton, Sadeghin, & Gropman, 2013, Samango-Sprouse et al., 2014). External factors include prenatal versus postnatal diagnosis (Girardin et al., 2009; Ross et al., 2012; Simpson et al., 2003), ancillary services received, and androgen replacement therapy implemented during infancy and/or at the onset of puberty (Mehta & Paduch, 2012; Patwardhan, Eliez, Bender, Linden, & Reiss, 2000; Ross et al., 2005; Samango-Sprouse, Sadeghin, et al., 2013; Samango-Sprouse et al., 2015).

Boys who are prenatally diagnosed are more likely to receive earlier ancillary services and/or hormonal treatment when there is a greater degree of neural plasticity, and positive response to treatment (Ross et al., 2017). Linden and Bender (2002), for instance, demonstrated a decrease in neurodevelopmental problems, particularly learning disabilities, in prenatally diagnosed as compared to postnatally diagnosed children with X and Y Chromosomal disorders.

In this study, we investigated two populations of boys with 47, XXY: one in the United States (US) and one in the Netherlands (NL). We examined the neurocognitive and behavioral profiles of these 47,XXY populations and assessed variability within them. We investigated how stable or "penetrant" the phenotype of 47,XXY is, and aimed to identify external factors that contribute to the observed phenotypic variability. These findings have theoretical relevance, in terms of both vulnerability mechanisms as well as clinical treatment in 47,XXY. A predictive algorithm detailing the most prominent contributors and their respective effect size on presentation could be very valuable in providing more personalized and targeted treatment options given the wide range of developmental function in 47,XXY.

2 | MATERIALS AND METHODS

2.1 | Participants

In the NL, 44 boys with 47,XXY participated as part of a large study on cognition and behavioral constructs in this disorder. The participants ranged from 8 to 18 years (mean 11.8 ± 3.5). The subjects included children with a prenatal diagnosis, children recruited through support groups, and those referred due to known developmental deficits. In the US, 54 boys with 47,XXY participated with ages ranging from 8 to 18 years (mean 11.5 ± 2.6). The group of US boys with 47,XXY were recruited through a diagnostic center as part of their routine neurodevelopmental care.

NL and US ages were not significantly different (p =.61). The presence of an extra X chromosome (non-mosaic) was confirmed using standard chromosomal testing. Informed consent was obtained from each study participant according to the Declaration of Helsinki. The Dutch Central Committee on Research Involving Human Subjects and the Western Institutional Review Board (WIRB) approved the study protocol (#20081226).

2.2 | Intellectual functioning

IQ profiles were measured using the English and Dutch versions of the Wechsler Intelligence Scales for Children (WISC) (Wechsler, 1991), and the Wechsler Intelligence Scales for Adults (WAIS) for study participants over 16 years of age (Wechsler, 2005). As in the original versions, the Dutch versions have adequate reliability and validity. The WISC generates a verbal IQ (VIQ), and a performance IQ (PIQ) (M = 100, SD = 15) through 12 subtests. VIQ assesses verbal skills such as verbal reasoning and verbal comprehension. PIQ assesses various visual-spatial abilities and perceptional reasoning.

2.3 | Parental education

Parental education was assessed according to the criteria of Hollingshead, employing a scale from 0 (no formal education) to 7 (graduate/professional training) (Hollingshead, 1975). Other scores of this scale include: 1 (less than seventh grade), 2 (junior high school), 3 (partial high school), 4 (high school graduate), 5 (partial college or specialized training), and 6 (standard college/university graduation) (Hollingshead, 1975). Level of education was averaged over both parents.

2.4 | Cognitive test battery

2.4.1 | Amsterdam Neuropsychological Tasks

The Amsterdam Neuropsychological Tasks (ANT) program (de Sonneville, 1999) has proven to be helpful in defining neurocognitive profiles in diverse populations (Rommelse et al., 2008; Serra et al., 2003) and several studies have demonstrated satisfactory psychometric properties of the ANT (for a review see de Sonneville, 2005). Stimuli are presented on the computer screen and participants respond using a mouse. Each subtest is preceded by illustration trials and practice trials. The ANT generates norm scores based on nonlinear regression functions capturing the normative data in the age range of 4–66 years with total sample sizes varying between 2,500 and 6,000 subjects, depending on type of task. Higher (positive) Z scores represent more impaired performance. A pilot study was performed in the US and showed that 15 typically developing children (aged between 8 and 18 years) had Z scores between Z − 0.5 and Z + 0.5 (i.e., well within the Dutch normal range). Based on this pilot study we were confident in using the Dutch norm scores for the US sample. Supplementary Table S1 provides an explanation of all subtests on the ANT.

2.4.2 | Emotional/behavioral problems

The norm-referenced Child Behavior Checklist (CBCL) describes recent emotional and behavioral functioning in children 6–18 years of age (Achenbach & Edlebrock, 1991). This assessment requires caregivers to fill out a checklist of behavioral or emotional problems exhibited by the child in the last two months. The items on the checklist are measured on a three point Likert scale. The CBCL has two empirically derived broadband scales (Externalizing and Internalizing) and eight syndrome scales (Anxious/Depressed, Withdrawn/Depressed, Somatic Complaints, Rule Breaking Behavior, Aggressive Behavior, Social Problems, Thought Problems, and Attention Problems). The Externalizing scale is comprised of items from the Rule Breaking and Aggressive Behavior syndrome scales; the Internalizing scale is comprised of items from the Anxious/Depressed, Withdrawn/Depressed, and Somatic Complaints syndrome scales. Raw scores for each scale are converted to norm-referenced *T*-scores ($M = 50$, SD = 10). The clinically relevant cutoff points of the CBCL broadband scores are $T < 60$ reflecting the normal range of functioning, $60 < T < 65$ reflecting the borderline range, and $T > 65$ reflecting the clinical range.

2.5 | Statistical analyses

Group comparisons of IQ and age (US/NL) were analyzed using Analysis of Variance (ANOVA), as these variables were normally distributed. Group comparisons of CBCL scores, parental education, number of children with prenatal/postnatal diagnosis, number of testosterone treated/non-treated children, number of children starting testosterone treatment in infancy versus childhood/adolescence, and the ANT parameters were analyzed using non-parametric (Mann–Whitney) tests, since these variables were not normally distributed. External factors, i.e., parental education, prenatal/postnatal diagnosis, and testosterone treatment (yes/no), were used to predict cognitive and behavioral scores using regression analyses, which do not require a normal distribution, in the US group and NL group, collapsed. Age was added to control for developmental effects. A hierarchical regression was used with age, parental education, pre/postnatal diagnosis, and testosterone treatment (yes/no) as predictors in the first box (stepwise selection). Country was added in the second box (remove selection) to test if, after explaining variance using parameters of interest in the first box, "country" was still a significant factor in explaining remaining variance (i.e., if it was required to improve the regression model). Because the number of children starting testosterone treatment in infancy versus childhood/adolescence was only applicable for the US sample, separate regression analyses were performed for this specific subgroup to predict cognitive and behavioral scores, with early versus late testosterone treatment and age as predictors (stepwise selection). Multicollinearity among predictors was also checked for in the regression analyses. For the group-wise (US/NL) nonparametric comparisons across multiple cognitive domains (ANT) and multiple behavioral domains (CBCL), the threshold for significance was set at $p = .01$ to correct for multiple comparisons. For other group comparisons that did not involve multiple comparisons, and for regression analyses, the threshold for significance was set at $p = .05$.

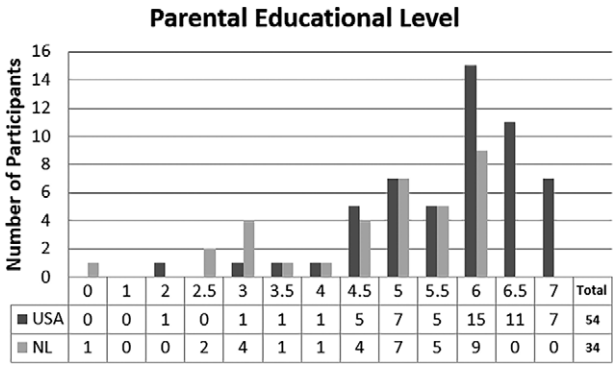

FIGURE 1 Average parental education level among the United States and Netherlands cohorts, as characterized by Hollingshead criteria

3 | RESULTS

3.1 | Group differences in environmental factors

3.1.1 | Parental education

Distribution of parental education scores in the US and NL samples are presented in Figure 1. Incomplete parental education data from 10 NL participants were removed. Average parental education was 5.7 (± 1.1) in the US and 4.6 (± 1.4) in the NL, which were significantly different ($z = -3.8$, $p < .001$). The higher score on parental education indicates more years of education. Average parental education was not different for testosterone treatment versus nontreated group, or for the prenatally diagnosed versus postnatally diagnosed group.

3.1.2 | Testosterone treatment

Distribution of testosterone treated versus nontreated in the US and NL samples are presented in Table 1. Of the NL children, 16.7% had received or were receiving testosterone treatment (vs. 83.3% non-treated children). Of the US children, 55.6% had received or were receiving testosterone treatment (vs. 44.4% non-treated). The group difference in ratio of testosterone treated and non-treated children was significant ($z = -3.8$, $p < .001$).

TABLE 1 This table presents the distribution of children who received testosterone treatment versus nontreated in the US and NL samples

	US	NL
Testosterone treatment	30	7
Nontreated	24	37
Prenatal diagnosis	49	24
Postnatal diagnosis	5	20

This table also presents the total number of participants diagnosed either prenatally or postnatally in the US and NL samples.

TABLE 2 *Z* scores on neuropsychological tests (ANT program) within the US and NL samples

Task	US	NL	Group differences
Attention regulation: Stability of tempo	1.16 ± .18	2.57 ± .32	$p = .001$*
Attentional control: Misses	1.74 ± .36	0.70 ± .26	$p = .03$
Inhibition	1.21 ± .27	1.28 ± .39	$p = .45$
Mental flexibility	1.64 ± .23	1.74 ± .39	$p = .54$
Motor fluency	1.22 ± .19	1.16 ± .16	$p = .59$
Motor flexibility	3.22 ± .49	2.10 ± .29	$p = .16$
Face processing	1.50 ± .41	1.18 ± .26	$p = .54$
Facial emotion recognition: Happy	0.81 ± .25	1.84 ± .20	$p = .03$
Facial emotion recognition: Sad	0.24 ± .23	0.90 ± .26	$p = .31$
Facial emotion recognition: Angry	0.92 ± .23	0.94 ± .24	$p = .73$

ANT, Amsterdam Neuropsychological Tasks battery.
Higher scores indicate more impaired performance.
*Significant at ($p = .01$) threshold, corrected for multiple comparisons.

3.1.3 | Prenatal/postnatal diagnosis

Distribution of timing of diagnosis (prenatally vs. postnatally) in the US and NL samples are also presented in Table 1. Of the NL children, 54.5% were prenatally diagnosed (vs. 45.5% postnatally diagnosed). Of the US children, 90.7% were prenatally diagnosed (vs. 9.3% postnatally diagnosed). The group difference in ratio of pre/postnatally diagnosed children was significant ($z = -4.1$, $p < .001$).

3.2 | Group differences in intellectual and cognitive functioning

Incomplete data from two participants in the NL sample were removed. In the NL sample, average VIQ was 78.1 (± 13.7) and average PIQ was 85.1 (± 15.7). In the US sample, average VIQ was 109.3 (± 11.3) and average PIQ was 114.9 (± 12.1). Group differences in VIQ and PIQ were significant: F (1,94) = 148.8, $p < 0.001$ and F (1,94) = 110.6, $p < 0.001$, respectively.

A summary of group differences in cognitive functioning as measured by the ANT is presented in Table 2. First, when comparing the average *Z* scores across the US sample and NL sample, the majority of cognitive parameters did not show significant group differences. However, as compared to the US sample, the NL sample showed more severe impairments in attention regulation as expressed in stability of tempo ($z = -3.3$, $p < 0.001$).

Next, in order to assess developmental risk within both samples, we calculated the percentage of children in the "intact/borderline" range ($Z < 2.0$, i.e., below 98th percentile) and "significantly impaired" range ($Z > 2.0$, i.e., above 98th percentile) for each cognitive domain. Performance was categorized on an individual level for each cognitive domain (Table 3).

We also calculated the percentage of children (who completed at least 8 out of 10 tests on the ANT) showing various levels of severity in their cognitive phenotype, as expressed by the number of cognitive domains that were affected (Figure 2). In the US group, a majority of

TABLE 3 Percentages of children with intact and impaired scores on neuropsychological tests (ANT program) within the US and NL samples

	US sample		NL sample		
Subtest	Normal/borderline (%)[a]	Impaired (%)[b]	Normal/borderline (%)[a]	Impaired (%)[b]	Group differences
Attention regulation (stability of tempo)	77.1	22.9	42.9	57.1	$p = .001$*
Attentional control: Misses	70.8	29.2	81.0	19.0	$p = .27$
Inhibition	74.1	25.9	72.1	27.9	$p = .83$
Mental flexibility	64.8	35.2	64.3	35.7	$p = .96$
Motor fluency	79.2	20.8	79.6	21.4	$p = .94$
Motor flexibility	44.4	55.6	55.8	44.2	$p = .29$
Face processing	77.4	22.6	75.0	25.0	$p = .79$
Facial emotion recognition: Happy	75.0	25.0	56.4	43.6	$p = .08$
Facial emotion recognition: Sad	83.7	16.3	77.8	23.1	$p = .44$
Facial emotion recognition: Angry	75.8	24.2	74.4	25.6	$p = .89$

[a]$Z < 2.0$.
[b]$Z > 2.0$.
*Significant at ($p = .01$) threshold, corrected for multiple comparisons.

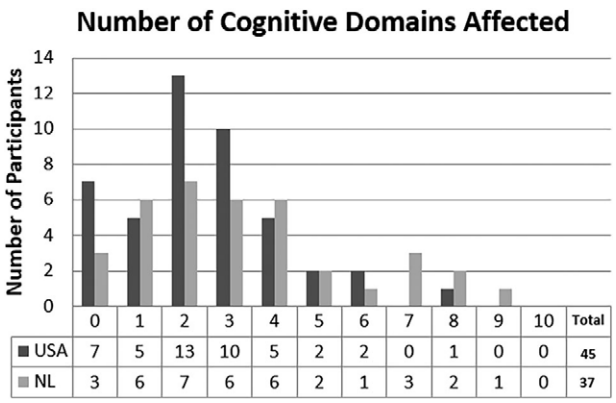

FIGURE 2 Number of cognitive domains affected among subjects in the United States and Netherlands cohorts, as measured by the Amsterdam Neuropsychological Tasks

the children (77.8%) had 0–3 cognitive domains affected, which suggests less effect of the additive X. There was only one US participant (2.2%) in the 7–10 cognitive domains affected range. In the NL group, the children were more evenly distributed across the range of cognitive domains affected. Of the NL children, 59.5% fell within the 0–3 cognitive domains affected, while 16.2% fell in the 7–10 cognitive domains affected range.

However, χ^2 analysis showed no significant differences in distribution between the US and NL groups ($z = -1.51$, $p = .13$). Also, the average percentage of affected domains (31.9% (± 23.9) for the NL sample and 28.4% (± 20.3) for the US sample) was not different across groups, ($z = -0.40$, $p = .68$).

3.3 | Group differences in emotional/behavioral problems

Incomplete CBCL data from eight participants in the NL sample were removed. Average CBCL T-scores for Total Problems were significantly higher in the NL sample (63.9 ± 9.6) than the US sample (54.8 ± 11.6), $z = -3.5$, $p < 0.001$. Similarly, scores for Internalizing Problems were significantly higher in the NL sample (63.4 ± 10.6) than the US sample (56.5 ± 11.7), $z = -2.74$, $p = .006$. Average *T*-scores for Externalizing Problems were 58.1 (±12.2) in the NL sample and 59.3 (± 11.7) in the US sample, which were not significantly different ($z = -2.4$, $p = .015$). See Table 4.

3.4 | Factors predicting intellectual, cognitive, and behavioral functioning

3.4.1 | Intellectual and cognitive functioning

For VIQ a significant model was found, F (3,83) = 20.9, $p < .001$, explaining 43.1% of the total variance in VIQ. This model included three significant predictors: parental education ($t = 3.9$, $p < .001$, $\beta = .34$), pre/postnatal diagnosis ($t = -4.6$, $p < .001$, $\beta = -.39$), and testosterone treatment ($t = 3.8$, $p < .001$, $\beta = .33$). Taken together, higher parental education, prenatal diagnosis, and testosterone treatment were significant predictors of higher VIQ.

A significant model was also found for PIQ, F (3,83) = 22.6, $p < .001$, explaining 45.0% of the total variance in PIQ. This model included three significant predictors: parental education ($t = 3.8$, $p < .001$, $\beta = .32$), pre/postnatal diagnosis ($t = -4.8$, $p < .001$, $\beta = -.40$), and testosterone treatment ($t = 4.4$, $p < .001$, $\beta = .36$). Higher parental education, prenatal diagnosis, and testosterone treatment were thus significant predictors of higher PIQ.

For Attention Regulation (stability of reaction times in the attention task) a significant model was found, F (2,77) = 6.6, $p = .002$, explaining 14.7% of the total variance in Attention Regulation. This model included two significant predictors: pre/postnatal diagnosis ($t = -2.4$, $p = .017$, $\beta = -.26$) and testosterone treatment ($t = -2.8$, $p = .006$, $\beta = -.30$). Prenatal diagnosis and testosterone treatment were thus significant predictors of better Attention Regulation.

Finally, for Motor Fluency, a significant model was found, F (1,83) = 4.9, $p = .029$, explaining 5.6% of the total variance in Motor Fluency. This model included one significant predictor: pre/postnatal diagnosis ($t = 2.2$, $p = .029$, $\beta = .24$). Prenatal diagnosis was thus a significant predictor of better Motor Fluency.

For each of the preceding independent variables (VIQ, PIQ, Attention Regulation, and Motor Fluency), the predictor "country," which was added in block two of the hierarchical regression, did not significantly improve the model.

No significant regression models were found for Face Processing, Emotion Recognition, Attentional Control, Mental Flexibility. For an

TABLE 4 Percentages of children with CBCL Problem Scales scores in the normal, borderline and clinical range within the US and NL Samples

	US			NL			
Problem Scale	Normal (%)[a]	Borderline (%)[b]	Clinical (%)[c]	Normal (%)[a]	Borderline (%)[b]	Clinical (%)[c]	Group differences
Total	70.4	7.4	22.2	36.1	16.7	47.2	$p = .002$*
Internalizing	63.0	7.4	29.6	38.9	8.3	52.8	$p = .02$
Externalizing	74.1	9.3	16.7	55.6	11.1	33.3	$p = .06$

CBCL, childhood behavioral checklist for ages 6–18
*Significant at ($p = .01$) threshold, corrected for multiple comparisons
[a]$T < 60$.
[b]$60 < T < 64$.
[c]$T > 65$.

overview of all significant predictors of intellectual and cognitive functioning, see Table 5.

Multicollinearity among predictors in the regression analyses revealed all variance inflation factor (VIF) values were approximately 1, demonstrating that these predictors (parental education levels, testosterone treatment, and time of diagnoses) are not intercorrelated. Thus, parental education was not associated with testosterone treatment or time of diagnoses.

3.4.2 | Behavioral functioning

For total level of emotional/behavioral problems (CBCL Total score) a significant model was found, F (2,84) = 7.8, p = .001, explaining 15.7% of observed variance. This model included two significant predictors: testosterone treatment (t = −3.0, p = .003, β = −.30) and pre/postnatal diagnosis (t = 2.7, p = .008, β = .27). The predictor "country," which was added in block two of the hierarchical regression, did not significantly improve the model. A prenatal diagnosis and testosterone treatment were significant predictors of fewer emotional/behavioral problems.

When looking at the two CBCL dimensions (Internalizing/Externalizing), a significant model was found for Internalizing Problems F (1,85) = 10.0, p = .002, explaining 10.5% of observed variance. For Internalizing Problems, one significant predictor was found: pre/postnatal diagnosis (t = 3.0, p = .002, β = .32). Also for CBCL Externalizing problems, a significant model was found, explaining 6.0% of the variance (F (1,85) = 5.4, p = .022). This model included one significant predictor: testosterone treatment (t = −2.3, p = .022, β = −.25). The predictor "country," which was added in block two of the hierarchical regressions, did not significantly improve the models. Prenatal diagnosis was thus a significant predictor of lower levels of internalizing problems, and testosterone treatment was a significant predictor of externalizing problems (Table 5).

With regard to the subgroups of testosterone-treated subjects (n = 42), some subjects began treatment in infancy (n = 16), while some subjects began treatment in childhood or adolescence (≥ age 6 years, n = 23). Therefore, within the testosterone treated sample, it was evaluated whether the variable "timing of treatment" (with the variable "age" added to control for maturational effects) predicted any of the cognitive and behavioral measures. The only cognitive/behavioral dimension that was significantly predicted by timing of treatment was Inhibition, which showed a significant model (F (1,47) = 5.0, p = .03) explaining 9.6% of the variance. This included one predictor which was timing of treatment (t = 2.2, p = .03, β = .31). Thus, testosterone treatment beginning in infancy, rather than childhood/adolescence, was associated with better inhibitory control. No significant models were found for the other cognitive/behavioral measures.

4 | DISCUSSION

To our knowledge, this is the first international and collaborative investigation using IQ assessment, a computerized neuropsychological

TABLE 5 Predictors of intellectual, cognitive, and behavioral outcome in the US and NL samples, collapsed

Test	Dependent variable	Total variance explained (R^2)	Significant independent variables (variance explained)	p-value
WISC	VIQ	43.1%	Parental Ed. (20.1%)	p < .001
WISC	VIQ	43.1%	Pre/post Dx (12.6%)	p < .001
WISC	VIQ	43.1%	Testosterone (10.5%)	p < .001
WISC	PIQ	53.5%	Parental Ed. (19.0%)	p < .001
WISC	PIQ	53.5%	Pre/post Dx (13.0%)	p < .001
WISC	PIQ	53.5%	Testosterone (13.0%)	p < .001
ANT	Attention regulation	14.7%	Testosterone (8.1%)	p = .006
ANT	Attention regulation	14.7%	Pre/post Dx (6.6%)	p = .017
ANT	Face processing	n.s		
ANT	Emotion recog. (Sad)	n.s.		
ANT	Emotion rec (Happy)	n.s.		
ANT	Attentional control (Misses)	n.s.		
ANT	Inhibition	9.6%	Timing of test. Treatment (9.6%)	p = .03
ANT	Mental flexibility	n.s.		
ANT	Motor fluency	5.6%	Pre/post Dx (5.6%)	p = .029
ANT	Motor flexibility	n.s.		
CBCL	Total	15.7%	Testosterone (8.3%)	p = .003
CBCL	Total	15.7%	Pre/post Dx (7.4%)	p = .008
CBCL	Internalizing	10.5%	Pre/post Dx (10.5%)	p = .002
CBCL	Externalizing	6.0%	Testosterone (6.0%)	p = .022

assessment, and parental questionnaires to further define the neurocognitive phenotype of boys with 47,XXY. Our findings suggest that verbal intelligence (VIQ), performance intelligence (PIQ), attention regulation, and degree of behavioral problems show significant differences across two samples, with a variety of critical factors contributing substantially to this divergence. These factors included parental education, time of diagnosis (prenatal/postnatal), as well as testosterone treatment. Our study provides insight into external factors that contribute to this variability in neuropsychological assessments, IQ measures, and problematic behavior observed in boys with 47,XXY. It also highlights some penetrant aspects of 47,XXY, as seen by the similar group scores on 9 out of the 10 ANT subtests. These findings are more robust given that the participants span two countries with diverse populations in terms of parental education, timing of diagnosis, treatment procedures, and interventional strategies. This study also provides an estimate of effect sizes on intellectual, cognitive, and behavioral outcome that has not been previously described.

Our findings are consistent with previous findings that have revealed boys with 47,XXY may exhibit deficits in executive functioning (Kompus et al., 2011; van Rijn & Swaab, 2015). Executive functioning is governed by the frontal lobe, and boys with 47,XXY have abnormal structure and function of this area of the brain (van Rijn & Swaab, 2015). Based on the findings in this study, it may be hypothesized that timing of diagnosis and testosterone treatment could influence brain function and neurodevelopment in a positive manner. Further, our results reveal that testosterone treatment beginning in infancy, rather than childhood/adolescence, was associated with better inhibitory control. These results further support previous studies suggesting that testosterone treatment may have a positive impact on neurodevelopment outcome in boys with 47,XXY (Mandoki & Sumner, 1991; Patwardhan et al., 2000; Ross et al., 2005; Ross et al., 2017; Samango-Sprouse, Sadeghin, et al., 2013).

Our findings suggest a neural plasticity to intellectual potential in boys with 47,XXY by three salient factors which are prenatal diagnosis, testosterone treatment, and parental education. Equally important, our findings support the need for systematic screening that could lead to prenatal diagnosis, improved parent support, and targeted early intervention. Based on our results, these proactive factors may minimize the negative impact of a prolonged diagnostic odyssey, optimize intellectual potential, and reduce behavioral disturbances.

Timing of diagnosis (prenatal versus postnatal) predicted the widest range of dependent variables—six in total (VIQ, PIQ, Attention Regulation, Motor Fluency, CBCL Total, CBCL Internalizing). This further solidifies the importance of timing of diagnosis and its significant impact on the neurodevelopmental outcome for boys with 47,XXY. Based on the results of this study and others, it is evident that comprehensive screening for the early diagnosis of 47,XXY would have a myriad of potential benefits for these children. Prenatal diagnosis allows for a proactive approach to care that includes early, dynamic treatment and the construction of a developmental infrastructure that addresses potential problems before their onset. Conversely, a reactive and variable care plan is more common in postnatally diagnosed children, who often do not receive appropriate services before the onset of problems.

Recently, several published articles have demonstrated the positive effect of testosterone on behavioral function and neurodevelopmental outcome (Mehta & Paduch, 2012; Patwardhan et al., 2000; Samango-Sprouse, Sadeghin, et al., 2013; Samango-Sprouse et al., 2015). Our current study adds further support for the potential effect of testosterone on verbal and perceptual reasoning, cognitive inhibition, and externalizing behaviors. Our findings suggest that some aspects of the neurodevelopmental disturbances of 47,XXY may be treatable by hormonal replacement, but further research is necessary on this relationship. Additionally, there may be a window of opportunity for optimal treatment, and additional study is warranted and underway on the optimal timing of hormonal treatment.

There is evidence that scores from the CBCL Internalizing scale may have the ability to discriminate between children with and without anxiety disorder (Seligman, Ollendick, Langley, & Baldacci, 2004). Behavioral dysfunction, such as anxiety disorders, low levels of testosterone, and high levels of estradiol, have been characterized in the phenotypic profile of 47,XXY as far back as the 1970's (Caldwell & Smith, 1972; Money, Annecillo, Orman, & Borgaonkar, 1974). In our study, prenatal diagnosis was the only significant predictor for decreased CBCL Internalizing problems, while testosterone was the only significant predictor for decreased CBCL Externalizing problems. Although this study did not find a relationship between testosterone and internalizing behavior problems, previous studies have shown a decrease in anxiety when boys with 47,XXY were treated with androgens during infancy, and between 6 and 12 years of age (Ross et al., 2017; Samango-Sprouse, Sadeghin, et al., 2013). More in-depth studies are warranted to further understand this interaction and relationship with hormonal production.

Results on the ANT assessment further expose the selective variability within the neurocognitive function associated with 47,XXY. Although individual variability was wide, the distribution was similar between the two international samples. Of note, the ANT subtest that showed significant group differences was Attention Regulation ($p = .001$), which was also the subtest that displayed the most variance due to external variables (Timing of Diagnosis and Testosterone). The lack of statistical significance for the other ANT subtests (Attentional Control, Inhibition, Mental Flexibility, Motor Fluency, Motor Flexibility, Face Processing, and Facial Emotion Recognition: Happy, Sad, Angry) suggests that these domains are more penetrant and less impacted by external variables. Taken together, these findings provide support for the need of targeted neuropsychological screening, treatment, and early detection for the boys with 47,XXY with this disorder.

In both samples, boys with 47,XXY were most vulnerable in motor flexibility with approximately half of the boys (US = 55.6%, NL = 44.2%) having impairments. Mental flexibility was also highly and consistently impacted, with 35.2% (US) and 35.7% (NL) of the children having dysfunctions in this domain. Our results support that the executive components of mental and motor flexibility may be more affected than previously appreciated. The flexibility component seems to be a rather penetrant aspect of the phenotype and was not significantly influenced

by testosterone treatment, parental education, or timing of diagnosis in either cohort. Given that 47,XXY boys are known to be at high risk for neurodevelopmental disturbances and executive dysfunction, comprehensive screening is warranted at specific times to identify and treat any neurodevelopmental dysfunction.

In our study, significant differences between samples in internalizing problem behavior were observed with time of diagnosis (prenatal/postnatal). Timing of diagnosis may play a role as this allows for early diagnosis, parental support, and psychosocial or pharmacological treatment. There is an overall lack of studies on early intervention in children with 47,XXY, and current findings call for further investigation. These findings suggest that the development of syndrome-specific and targeted treatment may be fruitful in reducing internalizing dysfunction and could be implemented across populations of boys with 47,XXY.

Further investigation is warranted in both populations to identify other factors indicating phenotypic vulnerability and, conversely, other potential positive contributors in boys with 47,XXY and/or their environment.

Limitations of the study may include different recruitment methods for NL and US participants and diverse care approaches in the two countries. Additionally, this study is inherently predisposed to ascertainment bias, as children who are referred to these centers for care may not be representative of the entire 47,XXY population, of which 75% never get diagnosed (Abramsky & Chapple, 1997; Bojesen et al., 2003).

5 | CONCLUSION

Our prospective study revealed that several external factors may play a significant role in understanding variability and penetrance in cognitive and behavioral outcome in 47,XXY, both within and across samples. Parental education, timing of diagnosis, and hormonal treatment all played an important role in neurocognitive performance. Identifying external factors in children with 47,XXY that may have a positive influence on outcome, such as timing of diagnosis, parental support, and testosterone treatment, is very important for improving clinical care. These findings provide a further foundation for the development of an outcome algorithm to identify vulnerable individuals with 47,XXY in order to provide them with care as soon as possible. Further studies are underway to identify and quantify other critical factors in infancy through early childhood in order to optimize the outcome for all boys with 47,XXY.

ACKNOWLEDGMENTS

We would like to express our deep gratitude to all our participants and their families from the Netherlands and United States. We would like to thank The Focus Foundation for their continued funding of our investigative studies. Lastly, we would like to thank the Netherlands Organization for Scientific Research for their VENI grant (number 016.095.060 to Sophie van Rijn) that helped to fund this study.

CONFLICTS OF INTEREST

The ANT program, used in this study, is commercially distributed by Sonares BV, Amsterdam, The Netherlands. Leo de Sonneville is the director of this firm. The rest of the authors declare that they have no competing interests.

ETHICS APPROVAL

All participants were consented prior to participation. For the Netherlands cohort, ethics approval was given by The Dutch Central Committee on Research Involving Human Subjects. For the United States cohort, ethics approval was given by the WIRB, protocol #20081226.

AUTHORS CONTRIBUTION

CS-S: study design, drafting of manuscript, editing manuscript, general oversight. ES: initial drafting of manuscript. PL: editing manuscript. SC: editing manuscript. TS: study design, general oversight. CC: editing manuscript. LdS: creator of one of the neuropsychological assessments used, statistical analyses. SvR: study design, editing manuscript, statistical analyses, general oversight.

ORCID

Carole Samango-Sprouse http://orcid.org/0000-0001-9941-0568

REFERENCES

Abramsky, L., & Chapple, J. (1997). 47,XXY (Klinefelter syndrome) and 47, XYY: Estimated rates of and indication for postnatal diagnosis with implications for prenatal counselling. *Prenatal Diagnosis*, *17*, 363–368.

Achenbach, T. M., & Edlebrock, C. (1991). *Manual for the Child Behavior Checklist Burlington*. VT: University of Vermont.

Boada, R., Janusz, J., Hutaff-Lee, C., & Tartaglia, N. (2009). The cognitive phenotype in Klinefelter syndrome: A review of the literature including genetic and hormonal factors. *Developmental Disabilities Research Reviews*, *15*, 284–294.

Bojesen, A., Juul, S., & Gravholt, C. H. (2003). Prenatal and postnatal prevalence of Klinefelter syndrome: A national registry study. *The Journal of Clinical Endocrinology & Metabolism*, *88*, 622–626.

Bruining, H., van Rijn, S., Swaab, H., Giltay, J., Kates, W., Kas, M. J., … de Sonneville, L. (2010). The parent-of-origin of the extra X chromosome may differentially affect psychopathology in Klinefelter syndrome. *Biological Psychiatry*, *68*, 1156–1162.

Caldwell, P. D., & Smith, D. W. (1972). The XXY (Klinefelter's) syndrome in childhood: Detection and treatment. *The Journal of Pediatrics*, *80*, 250–258.

de Sonneville, L. M. J. (1999). Amsterdam neuropsychological tasks: A computer-aided assessment program. *Computers in Psychology*, *6*, 187–203.

de Sonneville, L. M. J. (2005). Amsterdam neuropsychological tasks: Scientific and clinical applications. *Tijdschrift voor Neuropsychologie*, *1*, 27–41.

Girardin, C. M., Lemyre, E., Alos, N., Deal, C., Huot, C., & Van Vliet, G. (2009). Comparison of adolescents with Klinefelter syndrome according to the circumstances of diagnosis: Amniocentesis versus clinical signs. *Hormone Research in Paediatrics*, *72*, 98–105.

Hollingshead, A. B. (1975). Four-factor index of social status. Unpublished manuscript, Department of Sociology, Yale University.

Iitsuka, Y., Bock, A., Nguyen, D. D., Samango-Sprouse, C., Simpson, J. L., & Bischoff, F. Z. (2001). Evidence of skewed X-chromosome inactivation in 47,XXY and 48, XXYY klinefelter patients. *American Journal of Medical Genetics*, *98*, 25–31.

Kompus, K., Westerhausen, R., Nilsson, L. G., Hugdahl, K., Jongstra, S., Berglund, A., . . . Savic, I. (2011). Deficits in inhibitory executive functions in Klinefelter (47,XXY) syndrome. *Psychiatry Research*, *189*, 135–140.

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*, 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*, 119–129.

Linden, M. G., & Bender, B. G. (2002). Fifty-one prenatally diagnosed children and adolescents with sex chromosome abnormalities. *American Journal of Medical Genetics*, *110*, 11–18.

Mandoki, M. W., & Sumner, G. S. (1991). Klinefelter syndrome: The need for early identification and treatment. *Clinical Pediatrics*, *30*, 161–164.

Mehta, A., & Paduch, D. A. (2012). Klinefelter syndrome: An argument for early aggressive hormonal and fertility management. *Fertility and Sterility*, *98*, 274–283.

Mohd Nor, N. S., & Jalaludin, M. Y. (2016). A rare 47 XXY/46 XX mosaicism with clinical features of Klinefelter syndrome. *International Journal of Pediatric Endocrinology*, *2016*, 1–4.

Money, J., Annecillo, C., Orman, B. V., & Borgaonkar, D. S. (1974). Cytogenetics, hormones and behavior disability: Comparison of XYY and XXY syndromes. *Clinical Genetics*, *6*(5), 370–382.

Nielsen, J., & Wohlert, M. (1991). Chromosome abnormalities found among 34910 newborn children: Results from a 13-year incidence study in Århus, Denmark. *Human Genetics*, *87*, 81–83.

Patwardhan, A. J., Eliez, S., Bender, B., Linden, M. G., & Reiss, A. L. (2000). Brain morphology in Klinefelter syndrome Extra X chromosome and testosterone supplementation. *Neurology*, *54*, 2218–2223.

Perwein, E. (1984). Incidence of klinefelter's syndrome. *Klinefeleter's Syndrome*. Berlin: Springer, (pp. 8–11).

Rommelse, N. N. J., Altink, M. E., Oosterlaan, J., Buschgens, C. J. M., Buitelaar, J., & Sergeant, J. A. (2008). Support for an independent familial segregation of executive and intelligence endophenotypes in ADHD families. *Psychological Medicine*, *38*, 1595–1606.

Ross, J. L., Kushner, H., Kowal, K., Bardsley, M., Davis, S., Reiss, A. L., . . . Roeltgen, D. (2017). Androgen treatment effects on motor function, cognition, and behavior in boys with Klinefelter syndrome. *The Journal of Pediatrics*, *185*, 193–199.

Ross, J. L., Roeltgen, D. P., Kushner, H., Zinn, A. R., Reiss, A., Bardsley, M. Z., . . . Tartaglia, N. (2012). Behavioral and social phenotypes in boys with 47, XYY syndrome or 47,XXY Klinefelter syndrome. *Pediatrics*, *129*, 769–778.

Ross, J. L., Samango-Sprouse, C., Lahlou, N., Kowal, K., Elder, F. F., & Zinn, A. (2005). Early androgen deficiency in infants and young boys with 47,XXY Klinefelter syndrome. *Hormone Research in Paediatrics*, *64*, 39–45.

Samango-Sprouse, C., & Rogol, A. (2002). XXY: The hidden disability and a prototype for an infantile presentation of developmental dyspraxia (IDD). *Infants & Young Children*, *15*, 11–18.

Samango-Sprouse, C., Sadeghin, T., Mitchell, F. L., Dixon, T., Stapleton, E., Kingery, M., & Gropman, A. L. (2013). Positive effects of short course androgen therapy on the neurodevelopmental outcome in boys with 47, XXY syndrome at 36 and 72 months of age. *American Journal of Medical Genetics Part A*, *161A*, 501–508.

Samango-Sprouse, C., Stapleton, E. J., Lawson, P., Mitchell, F., Sadeghin, T., Powell, S., & Gropman, A. L. (2015). Positive effects of early androgen therapy on the behavioral phenotype of boys with 47,XXY. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*, *169*, 150–157.

Samango-Sprouse, C., Stapleton, E. J., Mitchell, F. L., Sadeghin, T., Donahue, T. P., & Gropman, A. L. (2014). Expanding the phenotypic profile of boys with 47,XXY: The impact of familial learning disabilities. *American Journal of Medical Genetics Part A*, *164A*, 1464–1469.

Samango-Sprouse, C., Stapleton, E., Sadeghin, T., & Gropman, A. L. (2013). Is it all the X: Familial learning dysfunction and the impact of behavioral aspects of the phenotypic presentation of XXY? *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*, *163*, 27–34.

Seligman, L. D., Ollendick, T. H., Langley, A. K., & Baldacci, H. B. (2004). The utility of measures of child and adolescent anxiety: A meta-analytic review of the Revised Children's Manifest Anxiety Scale, the State-Trait Anxiety Inventory for Children, and the Child Behavior Checklist. *Journal of Clinical Child and Adolescent Psychology*, *33*, 557–565.

Serra, M., Althaus, M., de Sonneville, L. M. J., Stant, A. D., Jackson, A. E., & Minderaa, R. B. (2003). Face recognition in children with a pervasive developmental disorder not otherwise specified. *Journal of Autism and Developmental Disorders*, *33*, 303–317.

Simpson, J. L., de la Cruz, F., Swerdloff, R. S., Samango-Sprouse, C., Skakkebaek, N. E., Graham, J. M., & Hall, J. G. (2003). Klinefelter syndrome: Expanding the phenotype and identifying new research directions. *Genetics in Medicine*, *5*, 460–468.

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*, 309–318.

Wechsler, D. (1991). *Manual for the Wechsler Intelligence Scale for Children-(WISC-III)*. San Antonio, TX: Psychological Corporation.

Wechsler, D. (2005). *WAIS-III NL. Wechsler Adult Intelligence Scale WAIS-III* (3rd ed.). Dutch version. Manual. Amsterdam: Harcourt Test Publishers.

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*, 200–208.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Samango-Sprouse C, Stapleton E, Chea S, et al. International investigation of neurocognitive and behavioral phenotype in 47,XXY (Klinefelter syndrome): Predicting individual differences. *Am J Med Genet Part A*. 2018;176:877–885. https://doi.org/10.1002/ajmg.a.38621