ARTICLE

Positive Effects of Early Androgen Therapy on the Behavioral Phenotype of Boys with 47,XXY

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47, XXY occurs in up to 1 in 650 male births and is associated with androgen deficiency, neurodevelopmental delays, and atypical social-behaviors. Previously, we showed that young boys with 47, XXY who received early hormonal therapy (EHT) had significantly improved neurodevelopment. The objective of this follow-up study was to examine the effects of EHT on social behavior in boys with 47, XXY. The study consisted of boys prenatally diagnosed with 47, XXY who were referred for evaluations. Twenty-nine boys received three injections of 25 mg testosterone enanthate and 57 controls did not receive EHT. Behavioral functioning was assessed using the Behavior Rating Inventory of Executive Function, Social Responsiveness Scale, 2nd Ed., and the Child Behavior Checklist for Ages 6–18. The hypothesis that EHT may affect behavior was formulated prior to data collection. Questionnaire data was prospectively obtained and analyzed to test for significance between two groups. Significant differences were identified between group's scores over time in Social Communication ($P = 0.007$), Social Cognition ($P = 0.006$), and Total T-score ($P = 0.001$) on the SRS-2; Initiation ($P = 0.05$) on the BRIEF; and Externalizing Problems ($P = 0.024$), Affective Problems ($P = 0.05$), and Aggressive Behaviors ($P = 0.031$) on the CBCL. This is the third study revealing positive effects of EHT on boys with XXY. There was a significant improvements associated with the 47, XXY genotype in boys who received EHT. Research is underway on the neurobiological mechanisms, and later developmental effects of EHT.

KEY WORDS: XXY; Klinefelter syndrome; androgens; chromosomal disorders

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INTRODUCTION

47, XXY, also known as Klinefelter syndrome (KS), is the most common X and Y chromosomal variation, estimated to occur in 1 in 650 male births, and is characterized by hypogonadism, tall stature, gynecomastia, and eunuchoidism [Klinefelter Jr et al., 1942; Maclean et al., 1961; Perwein, 1984; Nielsen and Wohlert, 1991; Bojesen et al., 2003; Aksglaede et al., 2008; Verri et al., 2010]. Neurodevelopmental delays and

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cognitive deficits, although varying in severity, are common in children with 47, XXY and include language- based learning difficulties, executive dysfunction, and speech delay [Graham et al., 1988; Ratcliffe, 1999; Samango-Sprouse and Rogol, 2002; Simpson et al., 2005; Kompus et al., 2011; Verri et al., 2010; Gropman & Samango-Sprouse, 2013]. These, in turn, are thought to contribute to the complex social and behavioral phenotype in school-age children with 47, XXY that may include both internalizing (e.g., – anxiety, social isolation) and externalizing (e.g., – aggression) behaviors and atypical social and peer interactions [Simpson et al., 2003; van Rijn et al., 2008; Bruining et al., 2009; Bishop et al., 2011; Ross et al., 2012; Samango-Sprouse et al., 2014].

Androgens, specifically testosterone, are known to have a broad influence on neurological development, cognitive functioning, and social behavior in males beginning in- utero and continuing through adulthood [Arnold and Breedlove, 1985; Knickmeyer and Baron-Cohen, 2006; Genazzani et al., 2007]. There are two testosterone surges that affect male neurodevelopment before puberty: an intrauterine surge occurring between 8 and 24 weeks gestation and the neonatal surge which begins two weeks after birth and continues until at least twenty-four weeks [Forest et al., 1973; Beck-Peccoz et al., 1991; Finegan et al., 1992]. This surge during infancy (also called "mini-puberty") is known to have profound effects on brain development, masculinzation of the infant boys as well as effect play, social interactions on male infants [Sørensen et al., 1981]. These early androgens, both during prenatal development and early infancy, influence gray matter volume as well as cortical maturation, which have been shown to have an organizational effect on social behaviors, cognitive abilities, language function, anxiety and fear reactivity during childhood and adolescence [Knickmeyer and Baron-Cohen, 2006; Bergman et al., 2010; Raznahan et al., 2010; Lombardo et al., 2012; Nguyen et al., 2013].

While androgen deficiency (and the positive impact of testosterone replacement) in adolescents and adults with 47, XXY has been well characterized, much less is known about the levels and effects of early androgens in children with 47, XXY [Hier and Crowley Jr, 1982; Lanfranco et al., 2004; Wikström et al., 2006; Aksglaede et al., 2009]. Ratcliffe et al. (1994) documented comparable fetal levels of testosterone in XXY males and XY controls between 16 and 20 weeks gestation; however, the window of opportunity to document androgen deficiency in utero may be small, as androgens may begin to rise as early as 9 weeks gestation, peak between 11 and 15 weeks, and begin to decline by 17 weeks [Ratcliffe et al., 1994; Finegan et al., 1989]. Two recent studies have observed lower levels of circulating androgens and a diminished postnatal surge in androgens in infants and young boys with 47, XXY [Lahlou et al., 2004; Ross et al., 2005]. There have also been several studies documenting low muscle tone, small testes and reduced phallic size reflective of early androgen deficiency in infants with 47, XXY [Lahlou et al., 2004; Ross et al., 2005; Zeger et al., 2008; Radicioni et al., 2010].

We hypothesized that the EHT may actually "prime the pump" of the androgen receptors in boys with XXY, which would then result in improvement in subsequent neurodevelopmental performance in the treated boys by supplementing their infantile androgen deficiency. We showed that children with 47, XXY who received short-course androgen therapy for diminished phallus size during infancy and prior to 15 months, had significantly improved cognitive functioning, visual- motor skills, and language development compared to 47, XXY controls who did not receive early hormonal therapy (EHT) [Samango-Sprouse et al., 2013b]. The positive impact of EHT on neurocognitive development has also been documented in a cohort of boys with 49, XXXXY [Samango-Sprouse et al., 2011]. Given the impact of early androgens on neurodevelopment and cognitive function, we hypothesize that boys with 47, XXY who received early androgen replacement therapy may also have significantly improved social skills and behavioral functioning in comparison to 47, XXY boys who did not receive treatment.

METHODS

Study Subjects

The study population consisted of 86 boys who were prenatally diagnosed with 47, XXY. These patients were from the same cohort of boys tested at 36 and 72 months of age in order to more comprehensively examine the possible longitudinal effects of EHT from early infancy throughout childhood. Patients were referred to the Neurodevelopmental Diagnostic Center in Davidsonville, MD, specializing in the neurodevelopment assessment of children with genetic disorders. Referrals were made from across the United States and neurodevelopmental evaluations were performed from 2009 to 2013. The Focus Foundation, a non-profit research organization for X and Y chromosome variations, provided funding to families that could not afford the evaluations in order to minimize ascertainment bias. Medical records were obtained for each patient that confirmed the 47, XXY diagnosis via karyotype and that documented the administration of any hormonal replacement.

Patients were referred by their primary care physician, their clinical geneticists, or were self-referred by parents. Patients were then evaluated by pediatric endocrinologists throughout the country. Twenty-nine of the referred patients received one intramuscular (IM) injection of 25mg testosterone enanthate once a month for three months for diminished phallic size. This IM dosage of testosterone has been shown to be effective for increasing penis size in infants and children [Guthrie et al., 1973; Bin-Abbas et al., 1999]. The timing of testosterone injections was determined on an individual basis and ranged from 4 to 15 months. No additional testosterone injections were given to any patient after this time and hormonal levels were not

typically obtained before or after these initial injections.

Fifty-seven boys with 47, XXY who did not receive any testosterone replacement therapy served as controls and were selected based on similarity with the EHT group's mean age and parent's highest level of obtained education (reflective of socio-economic status). Parental education was coded from 0 to 7 consistent with Hollingshead Four Factor Index of Socio Economic Status (SES) with 7 = graduate/professional training, 6 = standard college or university graduation, 5 = partial college, at least one year of specialized training, 4 = high school graduate, 3 = partial high school, 10th or 11th grade, 2 = junior high school, including 9th grade, 1 = less than 7th grade, 0 = not applicable or unknown [Hollingshead, 1975].

The two groups of subjects had similar number of visits for neurodevelopmental evaluation (treated group avg. = 10.2; untreated group avg. = 9.2) between time of diagnosis and 108 months. All families were asked to complete behavioral questionnaires at each visit and were unaware of which forms were used for this research study. All boys were referred for early intervention services including PT, OT and Speech and Language services as needed. The majority of boys in both groups continue to receive clinical care at our center.

Evaluations

Parental consent was obtained for each study participant. This included a detailed description of the study protocol approved by the Western Institutional Review Board (WIRB). Standardized testing was selected based on the subject's chronological age and included the Behavior Rating Inventory of Executive Function (BRIEF), Child Behavior Checklist for Ages 6–18 (CBCL), and the Social Responsiveness Scale, Second Edition (SRS-2). Examiners and scorers of the standardized assessments were blinded to which boys with XXY who had received EHT.

Statistical Analyses

Neurodevelopmental data was divided into two groups based on those infants who received androgen treatment (group 1) and those who did not (group 2). All test scores within the appropriate age range for each neurodevelopmental subtest were retrospectively obtained and used in the analyses. If multiple scores for an individual patient on a given subtest were available, all test scores falling within the appropriate age range for that subtest were used in the analyses. Test scores for each patient were de- identified according to the WIRB-approved protocol and an off-site biostatistician who had no interactions with patients performed all analyses.

Random mixed-effects models were used to determine significant differences between group scores taking into account the dependencies between repeated observations for each subject. The results produced in the random mixed-effects model is a linear regression model accounting for both within-subject factors (i.e. – the repeated measurements at each visit) and between-subject factors (i.e., – between the group who received testosterone and the group that did not). This was done using STAT's 'xtreg' command, which uses a weighted average of the within-subject effects and the between-effects. Therefore this model accounts for the effects of the testosterone treatment as well as the effects of the subjects being measured at different points in time. Significant treatment-by-visit effects indicate whether the average change in the dependent variable (questionnaire scores) over the various visits is statistically different between the two groups.

Significant group differences were also estimated excluding multiple visits per individual. The evaluation for each individual that was closest to 108 months

TABLE I. Parent Demographics

	Group 1: Treated	Group 2: Untreated	*P*-value
Fathers			
Mean age ± STD			
	37.9 ± 5.2	37.9 ± 6.6	*0.97*
Age range			
	30–51	27–46	
% College degree			
	90%	77%	*0.23*
Mothers			
Mean age ± STD			
	37.4 ± 4.8	36.8 ± 5.4	*0.68*
Age range			
	28–49	27–45	
% College degree			
	95%	80%	*0.12*

of age was selected and used in the analyses. Significant differences between group's scores were determined using the two-sample *t*- test if normality was present and the Wilcoxon- Mann–Whitney test if normality was absent. The Skewness–Kurtosis test was used to assess normality of the groups.

The null hypothesis was that there would be no statistically significant differences between the mean scores of group 1 and group 2 on any behavioral assessment.

RESULTS

Parental demographic information for each group is presented in Table I. The mean maternal and paternal ages were similar and not statistically different between the two groups ($P > 0.05$). The majority of mothers and fathers in both groups had at least 1 year of specialized training at a post-secondary institution (Hollingshead education code ≥ 5). The percentage of post-secondary degrees in mothers and fathers was not statistically different between the two groups. Each group also represented an equal distribution of first, second and third-born children (results not shown) and the age of patients ranged from 9 years to 11 years in both groups of boys with 47, XXY.

On the SRS-2, the linear mixed effects model revealed a significant difference between the group that received testosterone treatment and the untreated group in social cognition ($P = 0.002$), social communication ($P = 0.001$), social motivation ($P = 0.004$), autistic mannerisms ($P = 0.005$), and total T- score ($P = 0.001$) (Table II). Additionally, there were significant differences between scores at various visits in social communication, social motivation, autistic mannerisms, and total T-score (all $P < 0.05$) indicating changes in social behavioral functioning over time (Table III). The treatment-by-visit effect was also significant in social communication, social motivation, social cognition and total T-score ($P < 0.01$) (Table III). The goodness of fit results using the Wald chi-squared test suggest that all the coefficients in the model are jointly statistically significant in each case except when social awareness is the dependent variable (Table III).

Initiation was the only skill assessed by the BRIEF that was significantly different between groups when both testosterone treatment and dependency between repeated observations were factored into the model ($P = 0.05$, Treatment*Visit, Table IV).

In the CBCL data, if only the effect of testosterone treatment is factored into the model, there is a significant difference in the scores between the treated and untreated groups for school behavior ($P = 0.01$) and social problems ($P = 0.03$) (Table II). When both the treatment group and number of visits are considered, the treated group has significantly higher scores over time at the $P < 0.10$ level and has significantly lower scores in somatic complaint, aggressive behavior, externalizing problems, and affective problems over time compared to the control group while differences in internalizing problems approached significance ($P = 0.105$) (Table IV).

The repeated measures model largely confirms the results for the non-repeated measures analysis (Table V). In some cases, examining the testosterone treatment by visit interaction allows for more significant findings for differences between the groups over time than seen in the non-repeated measures analysis. All statistical analyses, including significant and non-significant findings, can be found in supplemental table SI.

DISCUSSION

These results provide additional support for the positive and sustained effects of short-course androgen therapy on neurodevelopmental outcome previously observed in the same cohort of patients with 47, XXY at 36 and 72 months of age [Samango-Sprouse et al., 2013b]. In the previous study, boys with 47, XXY who received EHT had improved speech and language development, reading skills, verbal and non-verbal intellectual quotients, and neuromotor

TABLE II. Linear Mixed-Effect Model Results (Includes Repeated Observations)

Test	Treated group			Untreated group			
Subtest	Mean	SD	N	Mean	SD	N	*P*-value
SRS							
Total T-score	53.3	13.52	54	60.19	16.16	102	0.001[a]
Social communication	53.07	13.25	54	59.49	15.48	102	0.001[a]
Autistic mannerism	52.41	11.77	54	60.72	14.41	102	0.005[a]
Social cognition	52.74	13.22	54	60.74	16.42	102	0.002[a]
BRIEF							
Initiation	50.94	12.52	32	59.55	10.82	42	0.05[a]
CBCL							
School	42.06	10.74	34	35.92	9.06	49	0.01[a]
Social problems	53.98	4.93	41	62.19	9.5	53	0.03*

[a]Denotes significance at the 5% significance level.

TABLE III. Results of Random Mixed Effects Model for SRS

	Dependent variable					
Independent variable	Social awareness	Social cognition	Social communication	Social motivation	Autistic mannerisms	Total T-score
Testosterone treatment	−3.6437	−8.2512*	−9.8382*	−8.1500*	−9.1574*	−9.6338*
	(−1.16)	(−3.04)	(−3.4)	(−2.89)	(−2.8)	(−3.4)
Visit	0.1383	−0.9299	−1.6171*	−2.5397*	−1.9232*	−2.0973*
	−0.16	(−1.34)	(−2.12)	(−3.46)	(−2.16)	(−2.86)
Treatment*visit	1.3966	3.2560*	3.5293*	3.85324*	2.3010*	4.0713*
	−0.93	−2.75	−2.72	−3.09	−1.52	−3.26
Constant	52.2394*	60.7300*	61.2159*	61.6213*	62.391*	62.109*
	−26.33	−29.69	−30.15	−30.28	−29.02	−30.07
Regression statistics						
Number of obs	156	156	156	156	156	156
Number of groups	79	79	79	79	79	79
R- squared (within)	0.026	0.1103	0.1418	0.1743	0.084	0.1643
R- squared (between)	0.003	0.0154	0.0079	0.0003	0.0422	0.0067
R- squared (overall)	0.006	0.022	0.0286	0.0168	0.0723	0.0201
Wald chi2(3)	2.11	10.39	12.24	14.85	10.47	14.67
Prob > chi2	0.55	0.0155	0.0066	0.002	0.015	0.0021

These are the estimated coefficients for the independent variables and the constant with the z statistic in parentheses.
*Denotes significance at the 5% significance level.

planning and execution. There have been no adverse effects or negative health outcomes reported in our population of boys with 47, XXY who received early short-course androgen therapy.

In the present study, boys who received EHT had significantly fewer behavioral problems and improved social behavioral skills that have been commonly associated with the 47, XXY genotype. These include externalizing behavior problems, aggressive behaviors, schooling behavior and affective problems. Somatic complaints were also reported by the parents to be significantly reduced in the EHT group. Somatic symptoms such as headaches and stomach aches are common in children with anxiety, language-based learning disabilities and social communication difficulties [Beidel et al., 1991; Dorn et al., 2003]. Studies have also found children that frequently present with somatic complaints are at a higher risk for internalizing disorders and anxiety [Masi et al., 1999], which, in turn, can have an adverse effect on school performance and school behavior from childhood through adolescence [Honjo et al., 2001].

The most significant behavioral differences observed in this study between the EHT and non-EHT group were within social domains, including social cognition, communication and overall social problems. Given the atypical social behaviors associated with Autism Spectrum Disorders it is not surprising that boys who received EHT also had significantly fewer autistic mannerisms indicated by the SRS-2 questionnaire. Recent studies have found an increased incidence of Autism Spectrum Disorders in 47, XXY boys; however, several of these studies may be limited by small sample sizes, varying screening methods, and incomplete evaluation of family history of learning disabilities and comorbid psychosocial disorders [Bruining et al., 2009; Bishop et al., 2011; Samango-Sprouse et al., 2014]. This study suggests that early androgen therapy in boys who are androgen deficient may reduce the presentation of atypical social behaviors associated with Autism Spectrum Disorders in children with 47, XXY. Conversely, Baron-Cohen has proposed that higher levels of androgens prenatally may predispose boys to ASD [Baron-Cohen et al., 2005], however the interaction between androgens and social behavior is not well understood. Further research is warranted to determine what hormonal factors, if any, may mediate the incidence of ASD in conjunction with increased androgen production. Additionally, the levels of androgen production during a pregnancy with 46, XY and 47, XXY have not been well investigated either. It is intriguing to consider the neurobiological interaction between prenatal levels of androgens, XXY and neurodevelopmental outcome especially in social behavior and pragmatic language.

Children who had received EHT in this study were also reported to have better initiation skills compared to the untreated group. This executive functioning skill, involving the ability to independently initiate tasks, responses and problem- solving skills, is believed

TABLE IV. Results of Random Mixed Effects Model for CBCL

	Dependent variable					
Independent variable	School	Somatic complain	Social problems	Aggressive behavior	Externalizing problems	Affective problems
Testosterone treatment	9.123[a]	1.1817	−5.5216[a]	2.2837	2.8261	0.065
	−2.56	−0.37	(−2.15)	−0.8	−0.74	−0.02
Visit	1.244	2.0239	0.05532	1.625	2.2303	3.0029[a]
	−0.96	−1.46	−0.06	−1.35	−1.46	−2.44
Treatment[a]Visit	−2.1418	−3.213[b]	−0.4037	−3.4847[a]	−4.589[a]	−3.174[b]
	(−1.21)	(−1.73)	(−0.31)	(−2.16)	(−2.25)	(−1.93)
Constant	33.757	56.721	61.129	54.867	50.560	56.148
	−14.46	−25.05	−33.99	−27.46	−18.88	−26.14
Regression Statistics						
Number of obs	83	94	94	94	94	94
Number of groups	52	57	57	57	57	57
R-squared (within)	0.0445	0.0485	0	0.0693	0.0988	0.1126
R-squared (between)	0.1049	0.0509	0.1834	0.064	0.0464	0.0763
R-squared (overall)	0.0952	0.0992	0.2191	0.111	0.0913	0.1287
Wald chi2(3)	7.23	5.77	10.49	6.79	6.76	9.56
Prob > chi2	0.0649	0.1233	0.0149	0.0788	0.0798	0.0227

These are the estimated coefficients for the independent variables and the constant with the z statistic in parentheses.
[a]Denotes significance at the 5% significance level.
[b]Denotes significance at the 10% significance level.

to be subsumed under the frontal lobe function of the brain [Miyake et al., 2000]. Several MRI studies of males with XXY have revealed atypical features of lobe morphology, cortical thickness and gray and white matter development associated with deficits in executive function [Giedd et al., 2006; Giedd et al., 2007; Lenroot et al., 2009; Lee et al., 2011; Mueller et al., 2011]. In a large quantitative MRI study of 42 young boys with 47, XXY, cortical thinning was pronounced in the left inferior frontal, temporal, and inferior parietal lobes compared to 46, XY

TABLE V. Two-sample mean comparison results (excludes repeated observations)

Test	Treated group			Untreated group			
Subtest	Mean	SD	N	Mean	SD	N	*P*-value
SRS							
Total T- score*	52.59	9.96	29	60.3	16.28	57	0.062
Social communication	52.14	10.17	29	59.33	15.45	57	0.026
Autistic mannerism*	51.79	8.43	29	61.19	15.87	57	0.018
Social cognition*	52.52	11.51	29	60.21	16.05	57	0.044
BRIEF							
Initiation	53.05	12.29	20	58.97	10.73	30	0.078
CBCL							
School	41.61	10.64	18	35.06	9.2	32	0.039
Social problems*	54.58	4.92	24	61.44	8.99	34	0.001

P-value from Mann–Whitney Test.
*Not normally distrubted.

controls. Cortical maturation and gray matter volume in these areas are known to be influenced by testosterone levels throughout childhood and adolescence and have been linked to language development, mood regulation, and impulsivity in normally developing males [Binder et al., 2000; Giedd et al., 2007; Raznahan et al., 2010]

Although the neurobiological mechanisms of androgen replacement in children with 47, XXY is unclear, the presence of androgen receptors in multiple cortical regions of the brain and the impact of androgens on brain structure and development has been reported in several studies of 46, XY and 47, XXY adolescents and adults [Patwardhan et al., 2000; Giedd et al., 2006; Lenroot et al., 2009; Raznahan et al., 2010]. Patwardhan et al. [2000] found that men with 47, XXY who received testosterone treatment during puberty had increased grey matter volume in the left temporal lobe and improved verbal fluency, associated with this brain region, compared to XXY controls who did not receive treatment [Patwardhan et al., 2000]. Our results suggest that early androgen therapy (supplementing the diminished neonatal surge reported in XXY males) may have pervasive and sustained effects on multiple aspects of neurodevelopment similar to those that have been documented previously in testosterone- treated males with 47, XXY. Future research is required to determine the optimal timing, long-term effects and biological mechanisms of early hormonal treatment in children with 47, XXY.

There are limitations of this study, however, that should also be taken into consideration. The decision to receive early hormonal replacement was made on an individual basis exclusively between parents and their pediatric endocrinologist. Although the socio-demographic and educational information of families are similar in the two groups (reflective of the affordability, availability and decision to receive EHT) there may be confounding factors that we were unable to account for, resulting in the significant behavioral differences observed between the two groups. For example, the clinical consideration of testosterone based on phallic size may lead to selection bias, with the more androgen-deficient children (resulting in a smaller phallus) more likely to receive treatment. Penis size and androgen levels, particularly in the untreated group, were not typically reported in the medical records for us to test this. However, if boys who received treatment were preferentially selected based on smaller phallus size, one might expect this group to have more severe behavioral outcomes as a result of lower androgen levels compared to the untreated group. If this were the case, the significant behavioral improvements in the treated group would provide further support for the potential positive impact of early androgen replacement on behavioral development in boys with 47, XXY. Despite the strong associations between EHT and positive behavioral outcomes in this study, causal relationships are unable to be drawn, particularly given the retrospective design of this study. This further supports the need for continued research into the impact of possible early biological treatment interventions in boys with 47, XXY.

CONCLUSION

This is now the third study (two being within the same cohort of boys with 47, XXY and the other being in a cohort of boys with 49, XXXXY) to reveal positive effects of early androgen replacement on the neurodevelopment of boys with X- chromosome aneuploidies [Samango-Sprouse et al., 2011; Samango-Sprouse et al., 2013b]. This study reveals, for the first time, the reduction of several characteristic features including affective problems, aggression and atypical social behaviors in children with 47, XXY who received early testosterone therapy during infancy. Additional research is required to determine the neurobiological mechanisms, optimal timing and later developmental effects of early androgen replacement in boys with 47, XXY.

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