HHS Public Access

Author manuscript

J Pediatr. Author manuscript; available in PMC 2019 September 21.

Published in final edited form as:

J Pediatr. 2017 June ; 185: 193–199.e4. doi:10.1016/j.jpeds.2017.02.036.

Androgen Treatment Effects on Motor Function, Cognition and Behavior in Boys with Klinefelter Syndrome

Judith L. Ross, M.D.[1,2], **Harvey Kushner, Ph.D.**[3], **Karen Kowal, P.A.**[1,2], **Martha Bardsley, M.D.**[1,2], **Shanlee Davis, M.D.**[4], **Allan L. Reiss, M.D.**[5], **Nicole Tartaglia, M.D.**[4], **David Roeltgen, M.D.**[6]

[1]Thomas Jefferson University, Department of Pediatrics, Philadelphia, Pennsylvania, 19107

[2]A.I. DuPont Hospital for Children, Wilmington, Delaware, 19803, 19104, Shore Physicians Group, Somers Point, NJ 08244

[3]Biomedical Research Institute, Philadelphia, Pennsylvania, 19115

[4]University of Colorado School of Medicine, Dept. of Pediatrics, Aurora, Colorado 80045

[5]Department of Psychiatry, Stanford University, Palo Alto, CA 94305

[6]Department of Neurology, University of Pennsylvania, Philadelphia, Pennsylvania

Abstract

Objectives—To examine the effects of early low-dose androgen on motor, cognitive, and behavioral function in prepubertal boys with Klinefelter syndrome (47,XXY).

Study design—Double-blind trial of 84 boys, ages 4–12 years, randomized to oxandrolone (Ox, 0.06 mg/kg daily, N=43) or placebo (Pl, N=41) for 24 months. Standardized assessments were performed at baseline and every 12 months for 24 months evaluating motor, cognitive, and behavioral function.

Results—The 24 month outcomes were better in the Ox vs. Pl group on one of five primary endpoints (motor function/strength): Bruininks Visual-Motor scale (P=0.005), without significant differences between the 2 groups for the other 4 components. Secondary analyses suggested improvement in the Ox vs. Pl group in the Anxiety/Depression (P=0.03) and Social Problems (P=0.01) scales on the Child Behavior Checklist, Anxiety (P=0.04) on the Piers Harris Self Concept Scale, and Interpersonal Problems (P=0.02) on the Children's Depression Inventory, without significant differences in hyperactive or aggressive behaviors.

Conclusions—This double-blind, randomized trial demonstrates that 24 months of childhood low-dose androgen treatment in boys with KS benefited one of five primary endpoints (visual-motor function). Secondary analyses demonstrated positive effects of androgen on aspects of

AUTHOR FOR CORRESPONDENCE: Judith L. Ross, MD, Thomas Jefferson University, Sidney Kimmel Jefferson Medical College, Department of Pediatrics, 833 Chestnut St., Philadelphia, PA 19107 USA, Phone: +1 215 955 1648; judith.ross@jefferson.edu.

psychosocial function (anxiety, depression, social problems), without significant effects on cognitive function, hyperactive or aggressive behaviors.

Trial registration—ClinicalTrials.gov:

Keywords

Klinefelter Syndrome; 47,XXY Child; Androgen; Randomized Controlled Trial; Testicular function; Gonadal failure; United States; Longitudinal Studies; Sex Chromosome Disorders, sex chromosome aneuploidy; sex chromosome variations

Klinefelter syndrome (KS) (1), an underdiagnosed genetic disorder that occurs in 1/500–1000 males (2), is defined by the chromosome karyotype 47,XXY and has characteristic physical, cognitive, and behavioral phenotypes. The KS physical phenotype includes testicular failure (androgen deficiency) and tall stature (3). The neurocognitive phenotype includes language-based learning difficulties and impairments in motor function, working memory, executive function, and attention (3–5). Approximately 50–75% of boys with KS demonstrate a specific reading/language disability, and 60–86% require special education services (6, 7). The behavioral profile includes shyness, diminished self-esteem, increased anxiety, depression, and social problems (8–10). The potential contribution of early childhood androgen deficiency versus the second × chromosome to these features is not known.

Clinical evidence of early childhood androgen deficiency in boys with KS comes from reports of small testes and genitalia in infancy and childhood (11–13), as well as eunuchoidal body proportions, hypotonia, and decreased muscle mass (3). The question of whether or not testosterone is low during infancy and childhood among boys with Klinefelter syndrome is not resolved. Moreover, testosterone levels in blood in KS have been reported as low for age, low normal, or normal in childhood and adulthood (13–17), and one study of infants with KS reported elevated testosterone levels (18). Evidence of testosterone deficiency in this study's 4–12 year old cohort comes from recently published baseline testosterone levels, which were significantly lower than the mean for age and were below the lower limit of normal in almost half of subjects (19).

Because testosterone affects typical brain development in males, this early androgen deficiency in KS is likely to have an impact on motor and cognitive function and behavior. Muscle mass and strength, motor function and self image have been reported to improve with androgen replacement in adolescents and adults with KS (5, 20, 21) and in other populations (22, 23). In this randomized, placebo-controlled study, we aimed to restore normal childhood levels of androgen for two years in prepubertal boys with KS through treatment with a synthetic oral androgen (oxandrolone, Ox). Low-dose androgen supplementation in boys with KS has not, to our knowledge, been previously evaluated prospectively. We postulated that low-dose, physiological androgen replacement during childhood would improve the primary outcome, motor function/strength. Seconday analyses evaluated effects on cognition and psychosocial function.

METHODS

Participants were recruited from a broad geographic and socioeconomic distribution through the support of the advocacy organization AXYS/KS&A, by direct referral, and through the internet. Inclusion criteria were: karyotype diagnosis of Klinefelter syndrome (47,XXY and variants [48,XXXY, 48,XXYY), <50% mosaicism for 46,XY cell line, age 4–12 years, no evidence of spontaneous onset of puberty (testicular size ≤ 4 ml), and no treatment with androgen in the preceding year. Exclusion criteria for this study were karyotypes including 46,XX males and 47,XYY males, intellectual disability, defined as baseline verbal or nonverbal Differential Ability Scales – 2nd edition (DAS-II) cluster standard scores <70 (< −2SD), and the inability to complete the cognitive and behavioral evaluation. A total of 9 subjects (3 Ox and 6 Pl) were excluded from these analyses, secondary to intellectual disability.

The study (conducted 2007–2011) was approved by the Human Subjects Committee of Thomas Jefferson University (TJU) and was registered with ClinicalTrials.gov (). Written informed consent was obtained from parent(s)/guardian and assent from patients. Participants were randomly assigned to treatment group in a 1:1 ratio using computer-generated randomization. Study medications were secured and dispensed by the TJU research pharmacy. Participants and investigators were blinded to treatment group assignment.

The protocol-specified oxandrolone dose was 0.06 mg/kg/day, rounded to the nearest 2.5 mg, and Ox or placebo (Pl), for 24 months. A protocol-specified dose reduction schedule was used, whereby dose was reduced by 50% if: LDL cholesterol > 159 mg/dl, HDL cholesterol < 20 mg/dl, liver function test (SGPT) exceeding twice the upper limit of normal for the assay (>90 IU/L), Tanner 2 pubic hair in boys < 8 years of age, bone age advancement >12 months/6 month interval and bone age > chronologic age, and systolic or diastolic blood pressure > 95th percentile for age and sex. We assessed compliance by having families fill out dosing cards and by counts of dispensed and returned capsules at each visit.

Safety Measures

Safety was evaluated at each visit by history, physical examination, and laboratory analyses, and results have been published (19). An independent Data and Safety Monitoring Board (DSMB) reviewed annual interim analyses and included experts in statistics, endocrinology and pediatrics.

Study Assessments

Subjects were evaluated on outcome measures at baseline, 12, and 24 months. The standardized cognitive and behavioral evaluation was performed by trained psychometricians over 3–4 hours (Table I). Socioeconomic status (SES) was derived from the Hollingshead 2-Factor Index (24).

Statistical Analyses

Analyses were performed using SAS software (9.2, SAS Institute, Inc., Cary, NC). For baseline comparisons, we used t-tests for continuous variables and Fisher exact tests for dichotomous variables. For longitudinal changes, we used a mixed model of repeated measures analysis of covariance, with fixed effects of treatment group and 24 month visit, comparing the change from baseline at 24 months in the Ox and Pl groups and adjusting for baseline differences in values, age, and socioeconomic status. The 12 month results are part of this mixed model repeated measures analysis, but only P-values for the 24 month data are presented. Data are presented as mean±SD or as least squares mean±SE. Our primary analysis specified five primary outcomes from the Motor/Strength Domain including BOT subscales of (1) Visual Motor Control, (2) Upper Limb Speed, and (3) Strength, Hand Strength Dynamometer-dominant hand, and PANESS finger-dominant hand. For these 5 primary efficacy measures comprising the primary endpoint, P-values are provided and the alpha level for statistical significance was set at 0.05/5=0.01 (two-tailed). Secondary analyses included measures of cognitive and social/behavioural function. There was no prespecified plan for adjustment for multiple comparisons in the analysis of secondary outcomes, and alpha ≤0.05 (two-tailed) was considered to be statistically significant. To evaluate the baseline proportion of clinically significant scales (%impaired), scores were divided into clinically significant (t score ≥1.7 SD [≥67]) for the CBCL (25).

RESULTS

Enrollment for this study was from June 20, 2007 until August 31, 2009. A total of 93 boys enrolled (Ox, n=46 or Pl, n=47, Figure 1; available at www.jpeds.com), and 84 were deemed eligible for this study.

Karyotypes in the 84 included 81 47,XXY, 2 mosaic 47,XXY/46,XY, and 1 KS variant (X;Y translocation). Diagnosis was made prenatally or in infancy in 69%. Participants came from 31 U.S. states and Canada from a broad range of socioeconomic status and parental education levels. Prior psychiatric diagnoses included ADHD in 28% and Autism Spectrum Disorder in 11%.

The two groups (Ox vs. Pl) had similar baseline IQ and SES values, but the Ox group was significantly younger (P<0.01), (Table II). Results were therefore adjusted for age within the ANCOVA model. A total of 84/72 subjects completed the baseline/24 month cognitive evaluations (Figure 1). No subjects withdrew secondary to significant adverse events, and safety data will be reported in a future manuscript.

A total of 17 of 72 subjects (7 Ox, 10 PL) who completed the 24 month trial had received treatment with testosterone at various dosages and durations in infancy or early childhood for durations of less than 0.7 year. Those with previous exposure to androgen therapy (23.6%) did not differ with respect to physical or gonadal function outcomes (26).

BASELINE FINDINGS

Primary outcomes (Domain 1)

Baseline performance on the BOT was decreased compared with population means (by ~0.5–1 SD) and did not differ between groups (Table III; available at www.jpeds.com). Grip strength measured by Hand Dynamometer was in the normal range at baseline.

Secondary outcomes

Cognitive function and language (Domain 2)—Verbal and nonverbal DAS standard scores were generally in the normative range (±2SD) for both groups (Ox, Pl) at baseline (Table III). However, subjects with verbal or nonverbal DAS cluster scores < 70 [< −2SD]) were excluded.

Working Memory/Attention (Domain 3)—For the working memory tests (Digit span backward and verbal fluencies), baseline performance was on average in the normative range, and the groups did not differ significantly. For the attention test, the Conners Continuous Performance Test (CPT), baseline standard scores tended to be impaired (−1–1.5 SD), most severely for omissions and perseverations.

Psychosocial and Behavior Domain (Domain 4)

Child Behavior Checklist (CBCL)

At baseline, scores were within 2 SD of the population mean for many of the Behavioral domains. However boys with KS had increased baseline t-scores score ≥1.7 SD[≥67] for CBCL Behavior problems 33% (28/84), Social problems 29% (24/84), Attention problems 35% (29/84), and Withdrawn scales 25% (21/84).

Child scales: Affect and Behavior (Domain 4)

Piers-Harris Self Concept Scale, 2nd Edition, and Children's Depression Inventory (CDI)—Baseline results were within 1–2 SD of the population means.

TREATMENT EFFECTS

Dose reductions

A total of 6 Ox versus 0 Pl patients had dose reductions for HDL < 20 mg/dl; 13 Ox versus 9 Pl dose reductions for bone age advancement. No patients in either group had dose reductions for pubertal development, blood pressure elevation or change in liver function tests.

Primary Outcome Analysis (Domain 1)

This analysis included 5 measures of motor function/strength: BOT Visual-Motor Control, BOT Upper Limb Speed, BOT Strength, PANESS-dominant hand, Hand Dynamometer-dominant hand) (Table III). On one of the 5 measures, the BOT Visual-Motor Control subtest, which measures how well the child can coordinate small hand movements and visual responses, the Ox group had better scores than the Pl group at 2 years after controlling

for baseline differences and age (P<0.005, Figure 2). The other four measures of motor function/strength showed no statistically significant differences in changes at 24 months for the Ox versus Pl groups. Grip strength measured by Hand Dynamometer increased at 24 months for the Ox versus Pl group, without attaining statistical significance (P=0.06, dominant hand). Adding the fixed variable of prior testosterone treatment to the ANCOVA model was not significant for any of the primary endpoints in this study except strength in the dominant hand for Hand Dynamometer in the Ox group ([fixed effect for prior testosterone treatment, P=0.02], [ANCOVA model for 2 year change, P=0.047], [2 year standard score means±SD for no prior treatment vs prior treatment: Ox: 124±14 vs 130±14m, Pl: 118±13 vs 112±18]).

Secondary Outcomes Analyses

Cognitive function and language (Domain 2)—Verbal and nonverbal DAS standard scores did not differ at 24 months between the groups (Table III).

Working Memory/Attention (Domain 3)—For the working memory tests (Digit span backward and verbal fluencies) and for the attention test (Conners Continuous Performance Test (CPT), the groups did not differ significantly over the 24 months study duration (Table III).

Psychosocial and Behavior Domain (Domain 4)

Child Behavior Checklist (CBCL)

At 24 months, the Ox group showed significant improvement in the CBCL Anxious/ Depressed (P<0.03), and Social Problems scales (acts young, teased, not liked) (P<0.01) (Table III). Other CBCL subscales including Aggressive, Delinquent, or Sex Problems scales did not differ at 24 months between the treatment groups.

Child scales: Affect and Behavior

The Piers-Harris Self Concept Scale, 2nd Edition—For the Anxiety scale, the Ox group had significantly improved standard scores (better self esteem, P<0.04, ANCOVA) at 2 years, compared with the Pl group. Other Piers-Harris subscales did not differ at 24 months between the treatment groups.

Children's Depression Inventory (CDI)—The Ox group had significantly better outcomes at 24 months on the CDI Interpersonal Problems (not getting along with others, P=0.02, ANCOVA), without significant differences in the other scales.

DISCUSSION

In this randomized, double-blind, placebo-controlled clinical trial, we evaluated 84 boys with KS, ages 4–12 years, who were treated with oxandrolone or placebo for 2 years. Important findings at baseline include low performance on the BOT standardized test for motor skills and the Conners Continuous Performance Test for attention. Ox treatment for 24 months resulted in improved visual-motor performance, but did not demonstrate significant effects of androgen treatment on the other four co-primary motor function/

strength endpoints. There were positive effects of Ox treatment on several aspects of anxiety/depression and social functioning, without adverse effects on behavior. Ox treatment did not have significant effects on most aspects of cognition (general cognition, verbal skills, working memory).

Although testosterone deficiency in boys with KS remains an area of debate, support for androgen deficiency occurring earlier in childhood in boys with KS includes the frequent lack of the typical neonatal testosterone surge, and the low/low-normal testosterone levels in childhood (13, 27, 28). These lower testosterone concentrations are correlated with subsequent diminished testicular and penile growth (29), altered cortical maturation, and increased social behavior concerns (30). Prepubertal testosterone levels are often below the detection limit for most assays, and radioimmunoassays (most common method of measuring testosterone) overestimate testosterone levels in children (31). Thus, whether testosterone levels are low and whether hypogonadism is present in boys with KS is not yet resolved (15) Testosterone replacement in infants, children, and adolescents with KS is quite variable with a lack of evidence-based recommendations or generally accepted clinical practice guidelines (32, 33).

The androgen receptor (AR) knockout mouse model supports the notion that testosterone acts physiologically at low levels in childhood because adult male-typical behaviors require AR-mediated androgen signaling early in life (34). Androgen deficiency and selective impaired learning has also been reported in an XXY mouse model, and testosterone replacement improved psychosocial deficits (35). Testosterone has organizational effects on the brain, both in utero and throughout life. Exposure to specific sex steroids leads to sex differences in brain and behavior (36–38), brain volume and cortical thickness, and gray matter and white matter development (39) in animal and human models (40).

Motor dysfunction and impaired visual-motor integration are cardinal features in KS, as reflected in our baseline findings and described by other investigators (5). In this study, we observed a significant positive Ox effect on a measure of visual-motor control, but did not observe a significant impact of Ox treatment on other aspects of motor function or strength. Visual-motor integration is required in many activities of daily living and school performance, and this domain has been previously reported to be impaired in KS (5). It is important to note that visual-motor control worsened throughout the two-year study period in boys treated with PI, while Ox seemed to protect against this decline. Possible mechanisms related to the decline include more severe androgen deficiency and/or increasingly impaired executive function as boys with KS grow older.

There have been retrospective reports about the impact of testosterone replacement on cognitive and behavioral outcomes in KS. Nonrandomized testosterone replacement in infancy was associated with higher scores in intellectual, language and neuromotor skills measured at 3 and 6 years of age, (41) and testosterone supplementation in hypogonadal adolescents and adults was associated with improved verbal fluency (42, 43). We did not find differences in cognition or working memory with our selected measurement tools after 24 months of Ox treatment.

Multiple studies have demonstrated an increased risk of ADHD (Inattentive type) in boys with KS, with 34–36% meeting DSM-IV criteria for ADHD (44). In this study, Ox treatment for two years was not associated with positive or negative effects on attention.

In contrast to the limited effects of Ox on motor or cognitive function of boys with KS, we found modest positive effects of Ox on psychosocial functioning as reported by both parent and child. Retrospective studies of early testosterone treatment has been reported to be associated with fewer behavior problems and better social skills later in childhood (45–47). In the current study, parents of children in the Ox group reported improved CBCL social problems (acts young, teased, not liked) scores, and the children themselves reported improvements in Interpersonal Problems (CDI) and with less anxiety (Piers Harris). Importantly, both the parents and participants were blinded to their treatment status, therefore taken together, our study results support modest positive effects of androgen therapy on anxiety and social functioning.

The “standard of medical care” for initiating testosterone replacement therapy in KS has typically been after failure of initiation or sustained development of puberty. There are few options for lower dose androgen dosing in childhood. Typically, adult androgen replacement is given using intramuscular injections or alternative formulations available only in higher doses.. We chose to use a low-dose, orally administered synthetic androgen treatment, oxandrolone, which is FDA-approved and has been used safely in boys with delayed puberty for over 30 years (48). Oxandrolone acts at the level of the androgen receptor (AR), is an AR agonist in vivo, and affects androgen-responsive target tissues (49), but is less virilizing, less hepatotoxic, and less active at a cellular level, compared with testosterone (50). However, there are several limitations related to oxandrolone. First, clinical assays to quantify serum levels are not available, so the dose could not be titrated within a range. Second, because Ox is a nonaromatizable androgen (51), it may be less physiologic than testosterone, and the aromatization of testosterone to estradiol may have separate, specific effects on brain and behavior. Thus, oxandrolone may offer adjuvant rather than replacement therapy for some physiological and psychosocial symptoms in KS.

In this study, dose reductions occurred, due to our predetermined “hard stops.” However, use of a more potent or higher dose androgen, aromatizeable or not, may have favorably (or unfavorably) altered the outcomes. Finally, there are likely to be organizational effects of prenatal or early postnatal sex steroids, which may be a “window of opportunity” that cannot be reclaimed with either an aromatizable or non aromatizable androgen outside of that critical time.

Although this is a large randomized controlled trial, we may have been underpowered to detect clinically meaningful benefits of oxandrolone treatment. This may be especially true for assessments with reduced sample size based on a minimum age (e.g. child questionnaires excluding children <6 years old (33% at start)). The target enrollment was initially set at 150 subjects and the actual enrollment was 93 subjects. This reduced the power to detect statistically significant differences. However, our original power analysis was based on our previous research and the work of others, and it showed that we had > 90% power ($\alpha = 0.05$,

two-tailed) to detect significant androgen effects on working memory/executive function for the treatment group versus the placebo group, with n=20 in each group.

In addition, there may have been study bias based on how the KS was diagnosed in our study cohort. Early diagnosis of KS in childhood is difficult and the rate of diagnosis is extremely low in childhood; only 10% of cases are identified before puberty with a subsequent rate of ascertainment during lifetime of 25% (52). The low rate of timely diagnosis is likely due to the fact that many of the classical signs and symptoms of androgen deficiency become evident in adolescence. To achieve the goal of increased early diagnosis in KS, it is necessary to increase medical awareness of the disease and in particular to augment pediatricians' knowledge that pathognomonic clinical features of KS are often lacking in childhood, but a characteristic cognitive and behavioral pattern is commonly present (53).

In conclusion, 2 years' treatment with childhood low-dose Ox was associated with positive effects on visual-motor integration and psychosocial function, without affecting most other motor or cognitive outcomes. The convergence between the child and parent measures in domains of social function indicates the results were clinically significant and meaningful. Importantly, there was no increase in negative behaviors with Ox treatment. Dosage individualization based on protocol-defined criteria was a unique aspect of the present study.

These findings need to be further validated with longer-term studies. Early diagnosis, together with parental education, developmental interventions, and potentially earlier androgen replacement may contribute to improved outcomes in KS, particularly in reduction of the social-psychosocial challenges (32). Future studies linking hormonal and genetic mechanisms will increase our understanding of the pathogenesis of KS and will permit more targeted interventions.

Acknowledgments

We thank the patients and their families for their participation in this study.

Supported by the National Institute of Neurological Disorders and Stroke (NINDS) (NS050597). N.T. is supported by NINDS (K23NS070337). The authors declare no conflicts of interest.

Appendix

Description of cognitive and behavioral tests

1. Assessments of Motor Function/Strength

- **A.** The Bruininks-Oseretsky Test of Motor Proficiency (BOT)
- **B.** Physical and Neurological Evaluation for Soft Signs (PANESS)
- **C.** Hand Strength Dynamometer

2. Assessment of General Cognition

- **A.** Differential Ability Scales – 2nd edition (DAS-II)

Assessment of Working Memory/Attention

A. Digit Span Backward

B. Verbal Fluencies: (A Developmental Neuropsychological Assessment, NEPSY)

C. Conners' Continuous Performance Test (CPT-II) (5–18+ yrs)/Kiddie CPT (4–5 yrs)

Domain 4. Self-image and social function

Parent Questionnaires

A. **The Child Behavior Checklist** (CBCL)

Child Self-Report Questionnaires (completed by the child)

A. **Self-Concept.** The Piers-Harris Self Concept Scale (SCS), Second Edition (ages 7–18 years).

B. **Depression.** The Children's Depression Inventory (CDI)

Socioeconomic Status (SES)

SES was derived from the Hollingshead 2-Factor Index [20].]

Battery of Tests

Assessments of motor function, cognitive function, and working memory

Scores are expressed as standard scores with mean of 100 and standard deviation (SD) of 15, unless indicated otherwise. Higher scores imply better function.

Domain 1. Motor function/Strength

The tasks used to assess fine and gross motor skills included: (the 5 primary endpoints:_Paness finger, BOT visual-motor control, BOT upper limb speed, BOT strength, and hand dynamometer-dominant hand).

A. The Bruininks-Oseretsky Test of Motor Proficiency (BOT) [1]. This battery assesses the child's motor development and includes standard scores (mean=100, SD=15) and subtest scores and is normed for sex and age (4–14.5 years). Time: 60 minutes.

B. PANESS (Physical and Neurological Evaluation for Soft Signs)[2] assesses the time required to press thumb to four fingers 20 times for the dominant and nondominant hands, with age-specific norms (4–18 years). Time: 5 minutes.

C. Hand strength dynamometer assesses hand strength in the dominant and nondominant hands and includes standard (mean=100, SD=15) scores. Dominance was defined as performing 5/8 or more tasks with that hand. Normative data are available from subjects ages 5–14 years, according to sex [3]. Time: 10 minutes.

Domain 2. General Cognition and Language

Differential Ability Scales – 2nd edition (DAS-11)[4] provides an age and sex standardized assessment of intellectual functioning (General Conceptual Ability; GCA, similar to Intelligence quotient) in children ages 2–17 years (mean=100, SD=15). The Preschool form (ages 4–5 years) is divided into a Verbal Cluster (including 2 subtests) and a Nonverbal Cluster (including 2 spatial and 1 nonverbal reasoning subtests). The School Age form (ages 6–17) includes three clusters. The Verbal Cluster measures the child's ability to define words and to perform verbal reasoning tasks. The Nonverbal Reasoning Cluster measures the child's inductive and sequential reasoning abilities. The Spatial Cluster measures visuospatial construction ability, spatial memory, and spatial reasoning. The Nonverbal and Spatial Clusters are computed for children > 6 years. Time: 75 minutes.

Domain 3. Working memory/attention

A. Digit Span Backward [5] This task is normed for children ages 5–16. Time: 10 minutes.

B. Verbal Fluencies (A Developmental Neuropsychological Assessment, NEPSY Verbal Fluency subtest) [6]: Semantic fluency measures the number of words the child can name in the categories Food and Drink (ages 4–12), and phonemic fluency measures the number of words the child can name beginning with the letters F and S (ages 6–12). Time: 10 minutes.

C. Conners' Continuous Performance Test (CPT-II) [7] (5–18+ yrs)/Kiddie CPT (4–5 yrs) measures ability to maintain attention over an extended period of time with a computer task that flashes different letters or pictures repeatedly on the screen and requires child to press the space bar each time a specific letter or picture appeared. Time: 15 minutes.

Domain 4. Self-image and social function

Parent Questionnaires (completed by the accompanying parent)

Scores are expressed as t-scores with mean of 50 and SD of 10, unless indicated otherwise.

Lower scores imply better function and higher scores indicate more problem behaviors.

A. The Child Behavior Checklist (CBCL)[9] is a standardized measure of behavior problems and social competency normed for children ages 4–16 and was completed by one parent or guardian. The CBCL includes t-scores for 10 problem behavior areas and for 3 social competency areas (activities, social, and school). Higher scores indicate more problems, with the cutoff for the clinical range at t-score ≥ 67 [9]. The behavior problems scales include internalizing, externalizing, and total behavior domain scores. The 3 social competency scales are scored such that higher scores indicate better social competence. Reliability and validity for the CBCL is well established and the measure is widely used in child behavior studies.

Child Self-Report Questionnaires (completed by the child)

A. Self-Concept. The Piers-Harris Self Concept Scale (SCS), Second Edition [10] (ages 7–18 years) is a self-report measure of self-concept. Scoring provides a total standard score and scores on six subscales: Physical Appearance and Attributes, Freedom from Anxiety, Intellectual and School Status, Behavioral Adjustment, Happiness and Satisfaction, Popularity.

B. Depression. The Children's Depression Inventory (CDI)[11] is a widely used self-report measure for assessment of depression in children. Reliability, internal consistency and validity have been well-established. The CDI assesses cognitive, affective and behavioral signs of depression in children ages 6–17. Total CDI score reflects the presence of overall depressive symptoms. Additional measures include Negative Mood (symptoms of sadness, guilt, crying), Interpersonal Problems (symptoms related to not getting along with others, misbehaving), Ineffectiveness (symptoms focusing on difficulties with schoolwork, feelings of inferiority), Anhedonia (symptoms of feeling decreased pleasure and fun, sleep or appetite changes, feeling alone, worrying), and Negative Self Esteem (symptoms of self-dislike, feeling unloved, feeling unsure of the future).

References

1. Bruininks R, Bruininks-Oseretsky Test of Motor Proficiency, Examiner's Manual. 1978, Circle Pines, MN: American Guidance Service.
2. Close J, Paness Physical and Neurological Examination, selected tests, in ECDEU Assessment Manual for Psychopharmacology, Guy W, Editor. 1976, N.I.M.H. (in the public domain), Abbott Laboratories: Rockville,MD p. 383–406.
3. Instruction/Owner's Manual for Hand Dynamometer. 1986, Lafayette: Lafayette Instrument.
4. Elliott CD, Differential Ability Scales-Introductory and Technical handbook. 1983, San Diego, CA: Harcourt, Brace, Jovanovich.
5. Cohen M, CMS-Children's Memory Scale Manual. 1997, San Antonio: Harcourt Brace & Company.
6. Korkman M, K.U., Kemp S, NEPSY A Developmental Neuropsychological Assessment Manual. 1998, Boston: The Psychological Corporation.
7. Connors C, Conners' Continuous Performance Test. 2000, Toronto: Multi-Health Systems, Inc.
8. Turriff A, Levy HP, and Biesecker B, Factors associated with adaptation to Klinefelter syndrome: the experience of adolescents and adults. Patient Educ Couns, 2015 98(1): p. 90–5. [PubMed: 25239793]
9. Achenbach TA, Manual for the Child Behavior Checklist//4–18 and 1991 Profile. 1991, Burlington, VT: University of Vermont Department of Psychiatry.
10. Piers E, V., Manual of the Piers-Harris children's self-concept scale. 1969, Nashville, TN: Counselor Recordings and Tests.
11. Kovacs M.a.M.S., CDI Children's Depression Inventory Technical Manual Update. 2003, North Tonawanda, NY: MHS.
12. Reynolds CR and Richmond BO, What I think and feel: a revised measure of children's manifest anxiety. J. Abn. Child Psychology, 1978 6: p. 271–280.

References

1. Klinefelter H, Reifenstein EC, Albright F. Syndrome characterized by gynecomastia, aspermatogenesis, without A-Leydigism and increased excretion of follicle stimulating hormone. J Clin Endocr Metab. 1942;2:615–27.
2. Nielsen J, Wohlert M. Sex chromosome abnormalities found among 34,910 newborn children: results from a 13-year incidence study in Arhus, Denmark. Birth Defects Orig Artic Ser. 1990;26(4):209–23. [PubMed: 2090319]
3. Zeger MP, Zinn AR, Lahlou N, Ramos P, Kowal K, Samango-Sprouse C, et al. Effect of ascertainment and genetic features on the phenotype of Klinefelter syndrome. The Journal of pediatrics. 2008;152(5):716–22. [PubMed: 18410780]
4. Ross JL, Roeltgen DP, Stefanatos G, Benecke R, Zeger MP, Kushner H, et al. Cognitive and motor development during childhood in boys with Klinefelter syndrome. American journal of medical genetics. 2008;146A(6):708–19. [PubMed: 18266239]
5. Salbenblatt JA, Meyers DC, Bender BG, Linden MG, Robinson A. Gross and fine motor development in 47,XXY and 47,XYY males. Pediatrics. 1987;80(2):240–4. [PubMed: 3615095]
6. Walzer S X chromosome abnormalities and cognitive development: implications for understanding normal human development. J Child Psychol Psychiatry. 1985;26(2):177–84. [PubMed: 3884639]
7. Close S, Fennoy I, Smaldone A, Reame N. Phenotype and Adverse Quality of Life in Boys with Klinefelter Syndrome. The Journal of pediatrics. 2015;167(3):650–7. [PubMed: 26205184]
8. Denai MA, Mahfouf M, Ross JJ. A hybrid hierarchical decision support system for cardiac surgical intensive care patients. Part I: Physiological modelling and decision support system design. Artif Intell Med. 2009;45(1):35–52. [PubMed: 19112012]
9. Tartaglia N, Cordeiro L, Howell S, Wilson R, Janusz J. The spectrum of the behavioral phenotype in boys and adolescents 47,XXY (Klinefelter syndrome). Pediatr Endocrinol Rev. 2011;8 Suppl 1:151–9.
10. Bishop DV, Scerif G. Klinefelter syndrome as a window on the aetiology of language and communication impairments in children: the neuroligin-neurexin hypothesis. Acta Paediatr. 2011;100(6):903–7. [PubMed: 21418292]
11. Stewart DA, Bailey JD, Netley CT, Rovet J, Park E, Cripps M, et al. Growth and development of children with X and Y chromosome aneuploidy from infancy to pubertal age: the Toronto study. Birth Defects Orig Artic Ser. 1982;18(4):99–154.
12. Ratcliffe SG. The sexual development of boys with the chromosome constitution 47,XXY (Klinefelter's syndrome). Clin Endocrinol Metab. 1982;11(3):703–16. [PubMed: 7139994]
13. Ross JL, Samango-Sprouse C, Lahlou N, Kowal K, Elder FF, Zinn A. Early androgen deficiency in infants and young boys with 47,XXY Klinefelter syndrome. Horm Res. 2005;64(1):39–45. [PubMed: 16088206]
14. Ratcliffe SG, Bancroft J, Axworthy D, McLaren W. Klinefelter's syndrome in adolescence. Archives of disease in childhood. 1982;57(1):6–12. [PubMed: 7065696]
15. Cabrol S, Ross JL, Fennoy I, Bouvattier C, Roger M, Lahlou N. Assessment of Leydig and Sertoli Cell Functions in Infants with Nonmosaic Klinefelter Syndrome: Insulin-Like Peptide 3 Levels Are Normal and Positively Correlated with LH Levels. J Clin Endocrinol Metab. 2011;96(4):E746–53. [PubMed: 21307139]
16. Topper E, Dickerman Z, Prager-Lewin R, Kaufman H, Maimon Z, Laron Z. Puberty in 24 patients with Klinefelter syndrome. Eur J Pediatr. 1982;139(1):8–12. [PubMed: 6816603]
17. Bastida MG, Rey RA, Bergada I, Bedecarras P, Andreone L, del Rey G, et al. Establishment of testicular endocrine function impairment during childhood and puberty in boys with Klinefelter syndrome. Clinical endocrinology. 2007;67(6):863–70. [PubMed: 17645574]
18. Aksglaede LP JH, Main KM, Skakkebaek NE, Juul A High normal testosterone levels in infants with non-mosaic Klinefelter's syndrome. European Journal of Endocrinology. 2007;157:345–50. [PubMed: 17766718]
19. Davis S, Lahlou N, Bardsley M, Temple MC, Kowal K, Pyle L, et al. Gonadal function is associated with cardiometabolic health in pre-pubertal boys with Klinefelter syndrome. Andrology. 2016.

20. Hines M, Fane BA, Pasterski VL, Mathews GA, Conway GS, Brook C. Spatial abilities following prenatal androgen abnormality: targeting and mental rotations performance in individuals with congenital adrenal hyperplasia. Psychoneuroendocrinology. 2003;28(8):1010–26. [PubMed: 14529705]
21. Robinson A, Bender BG, Borelli JB, Puck MH, Salbenblatt JA, Winter JS. Sex chromosomal aneuploidy: prospective and longitudinal studies. Birth Defects Orig Artic Ser. 1986;22(3):23–71. [PubMed: 3814779]
22. Bhasin S, Storer TW, Berman N, Yarasheski KE, Clevenger B, Phillips J, et al. Testosterone replacement increases fat-free mass and muscle size in hypogonadal men. J Clin Endocrinol Metab. 1997;82(2):407–13. [PubMed: 9024227]
23. Crawford BA, Liu PY, Kean MT, Bleasel JF, Handelsman DJ. Randomized placebo-controlled trial of androgen effects on muscle and bone in men requiring long-term systemic glucocorticoid treatment. J Clin Endocrinol Metab. 2003;88(7):3167–76. [PubMed: 12843161]
24. Hollingshead AB, Redlich F. Social Class and Mental Illness. New York: John Wiley; 1958.
25. Achenbach TA. Manual for the Child Behavior Checklist//4–18 and 1991 Profile. Burlington, VT: University of Vermont Department of Psychiatry; 1991.
26. Davis SM, Cox-Martin M, Bardsley M, Kowal K, Zeitler PS, Ross JL. Effects of Oxandrolone on Cardiometabolic Health in Boys with Klinefelter syndrome: A Randomized Controlled Trial. J Clin Endocrinol Metab. 2016:jc20162904.
27. Wikstrom AM, Dunkel L. Testicular function in klinefelter syndrome. Horm Res. 2008;69(6):317–26. [PubMed: 18504390]
28. Lahlou N, Fennoy I, Ross JL, Bouvattier C, Roger M. Clinical and hormonal status of infants with non-mosaic XXY karyotype. Acta Paediatr. 2011.
29. Kuiri-Hanninen T, Seuri R, Tyrvainen E, Turpeinen U, Hamalainen E, Stenman UH, et al. Increased activity of the hypothalamic-pituitary-testicular axis in infancy results in increased androgen action in premature boys. J Clin Endocrinol Metab. 2011;96(1):98–105. [PubMed: 20881260]
30. Knickmeyer RC, Baron-Cohen S. Fetal testosterone and sex differences in typical social development and in autism. J Child Neurol. 2006;21(10):825–45. [PubMed: 17005117]
31. Moal V, Mathieu E, Reynier P, Malthiery Y, Gallois Y. Low serum testosterone assayed by liquid chromatography-tandem mass spectrometry. Comparison with five immunoassay techniques. Clinica chimica acta; international journal of clinical chemistry. 2007;386(1–2):12–9. [PubMed: 17706625]
32. Davis S, Howell S, Wilson R, Tanda T, Ross J, Zeitler P, et al. Advances in the Interdisciplinary Care of Children with Klinefelter Syndrome. Advances in pediatrics. 2016;63(1):15–46. [PubMed: 27426894]
33. Davis SM, Rogol AD, Ross JL. Testis Development and Fertility Potential in Boys with Klinefelter Syndrome. Endocrinol Metab Clin North Am. 2015;44(4):843–65. [PubMed: 26568497]
34. Sato T, Matsumoto T, Kawano H, Watanabe T, Uematsu Y, Sekine K, et al. Brain masculinization requires androgen receptor function. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(6):1673–8. [PubMed: 14747651]
35. Barnea-Goraly N, Menon V, Eckert M, Tamm L, Bammer R, Karchemskiy A, et al. White matter development during childhood and adolescence: a cross-sectional diffusion tensor imaging study. Cereb Cortex. 2005;15(12):1848–54. [PubMed: 15758200]
36. Geschwind DH, Gregg J, Boone K, Karrim J, Pawlikowska-Haddal A, Rao E, et al. Klinefelter's syndrome as a model of anomalous cerebral laterality: testing gene dosage in the X chromosome pseudoautosomal region using a DNA microarray. Dev Genet. 1998;23(3):215–29. [PubMed: 9842716]
37. Giedd JN, Clasen LS, Wallace GL, Lenroot RK, Lerch JP, Wells EM, et al. XXY (Klinefelter syndrome): a pediatric quantitative brain magnetic resonance imaging case-control study. Pediatrics. 2007;119(1):e232–40. [PubMed: 17200249]
38. Raznahan A, Lee Y, Stidd R, Long R, Greenstein D, Clasen L, et al. Longitudinally mapping the influence of sex and androgen signaling on the dynamics of human cortical maturation in

adolescence. Proceedings of the National Academy of Sciences of the United States of America. 2010.

39. Lombardo MV, Ashwin E, Auyeung B, Chakrabarti B, Taylor K, Hackett G, et al. Fetal testosterone influences sexually dimorphic gray matter in the human brain. J Neurosci. 2012;32(2): 674–80. [PubMed: 22238103]
40. Rasika S, Nottebohm F, Alvarez-Buylla A. Testosterone increases the recruitment and/or survival of new high vocal center neurons in adult female canaries. Proceedings of the National Academy of Sciences of the United States of America. 1994;91(17):7854–8. [PubMed: 8058723]
41. Samango-Sprouse CA, Sadeghin T, Mitchell FL, Dixon T, Stapleton E, Kingery M, et al. 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. 2013;161A(3):501–8. [PubMed: 23345253]
42. Patwardhan AJ, Eliez S, Bender B, Linden MG, Reiss AL. Brain morphology in Klinefelter syndrome: extra X chromosome and testosterone supplementation. Neurology. 2000;54(12):2218–23. [PubMed: 10881243]
43. Cherrier MM, Matsumoto AM, Amory JK, Johnson M, Craft S, Peskind ER, et al. Characterization of verbal and spatial memory changes from moderate to supraphysiological increases in serum testosterone in healthy older men. Psychoneuroendocrinology. 2007;32(1):72–9. [PubMed: 17145137]
44. Tartaglia NR, Ayari N, Hutaff-Lee C, Boada R. Attention-deficit hyperactivity disorder symptoms in children and adolescents with sex chromosome aneuploidy: XXY, XXX, XYY, and XXYY. Journal of developmental and behavioral pediatrics : JDBP. 2012;33(4):309–18. [PubMed: 22333574]
45. Turriff A, Levy HP, Biesecker B. Prevalence and psychosocial correlates of depressive symptoms among adolescents and adults with Klinefelter syndrome. Genet Med. 2011;13(11):966–72. [PubMed: 21799429]
46. Nielsen J, Pelsen B. Follow-up 20 years later of 34 Klinefelter males with karyotype 47,XXY and 16 hypogonadal males with karyotype 46,XY. Hum Genet. 1987;77(2):188–92. [PubMed: 3653891]
47. Samango-Sprouse C, Stapleton EJ, Lawson P, Mitchell F, Sadeghin T, Powell S, et al. 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. 2015;169(2):150–7.
48. Fox-Wheeler S, Heller L, Salata CM, Kaufman F, Loro ML, Gilsanz V, et al. Evaluation of the effects of oxandrolone on malnourished HIV-positive pediatric patients. Pediatrics. 1999;104(6):e73. [PubMed: 10586007]
49. Kemppainen JA, Langley E, Wong CI, Bobseine K, Kelce WR, Wilson EM. Distinguishing androgen receptor agonists and antagonists: distinct mechanisms of activation by medroxyprogesterone acetate and dihydrotestosterone. Mol Endocrinol. 1999;13(3):440–54. [PubMed: 10077001]
50. Hart DW, Wolf SE, Ramzy PI, Chinkes DL, Beauford RB, Ferrando AA, et al. Anabolic effects of oxandrolone after severe burn. Ann Surg. 2001;233(4):556–64. [PubMed: 11303139]
51. Orr R, Fiatarone Singh M. The anabolic androgenic steroid oxandrolone in the treatment of wasting and catabolic disorders: review of efficacy and safety. Drugs. 2004;64(7):725–50. [PubMed: 15025546]
52. Bojesen A, Juul S, Gravholt CH. Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. J Clin Endocrinol Metab. 2003;88(2):622–6. [PubMed: 12574191]
53. Messina MF, Sgro DL, Aversa T, Pecoraro M, Valenzise M, De Luca F. A characteristic cognitive and behavioral pattern as a clue to suspect Klinefelter syndrome in prepubertal age. Journal of the American Board of Family Medicine : JABFM. 2012;25(5):745–9. [PubMed: 22956710]

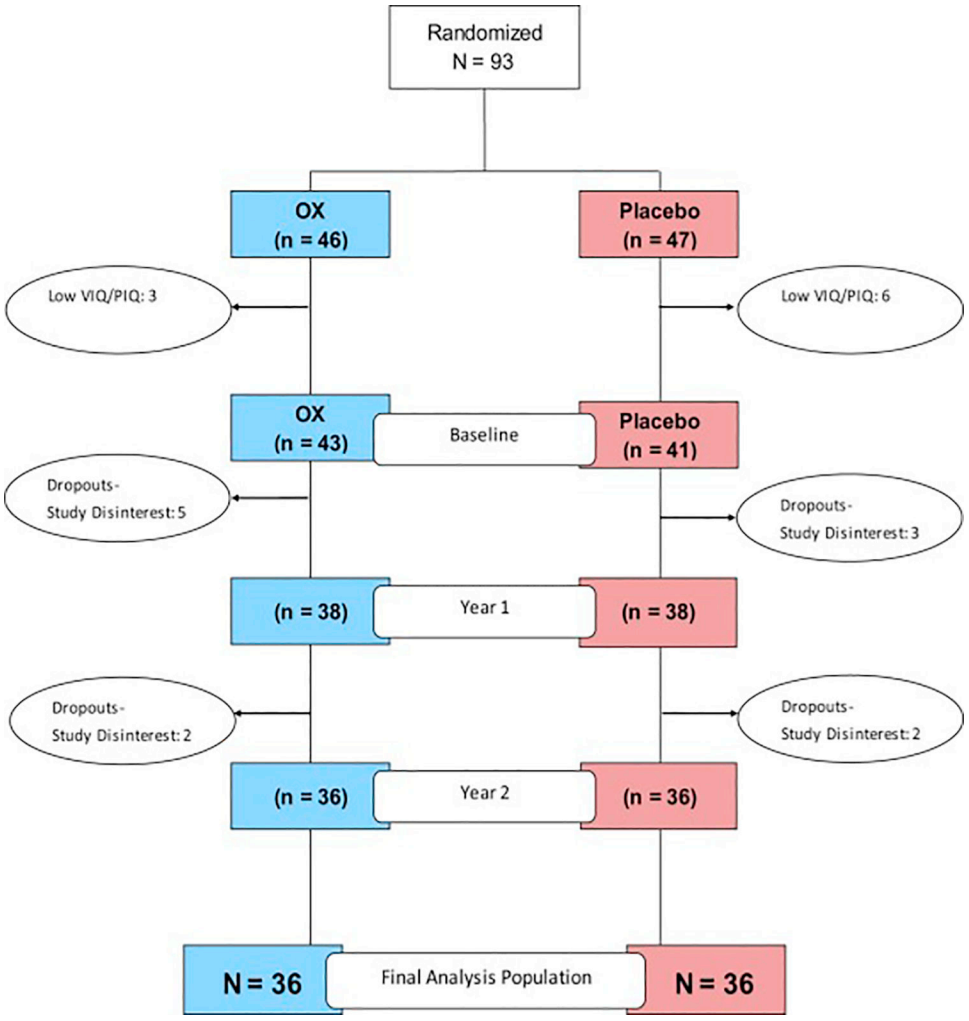

Figure 1: Study disposition.

A total of 93 boys were enrolled and randomized (Ox, n=46 or Pl, n=47), and 84 met study criteria. Participant demographics were 75% Caucasian, 1% African-American, 9% Hispanic, 5% Asian-American, and 10% Other. There were 12 dropouts (7 Ox, 5 Pl) (14% dropout rate). Reasons for study discontinuation included lack of interest in all 12. None withdrew because of adverse events or safety reasons.

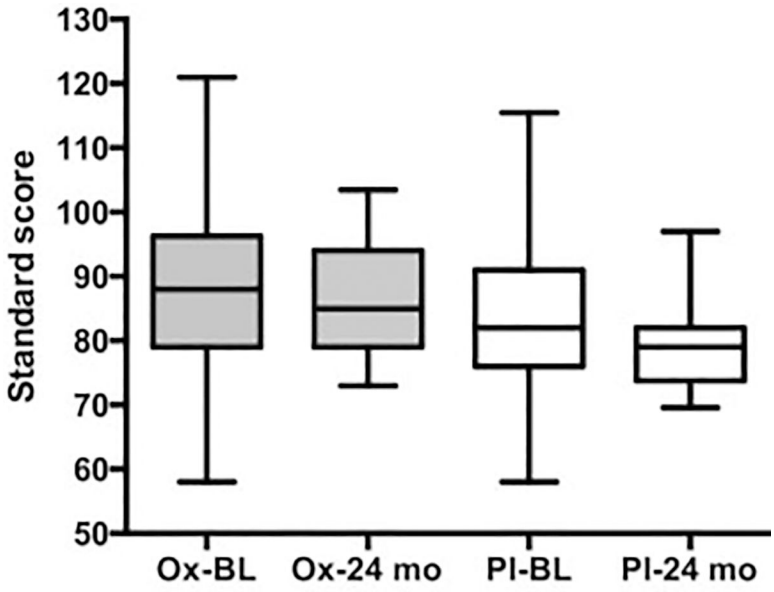

Figure 2: Effects of Oxandrolone treatment on BOT Visual Motor Control.

Box and whiskers plot of longitudinal baseline and 24 month scores for Ox (left) and Pl (right). The solid line in the box is the median, the box range is the 25^{th}-75^{th} %ile, and the whiskers up to the largest and go down to the smallest value.

Table 1:

Cognitive and behavioral evaluation

Primary Outcome analysis **Domain 1: Motor function/Strength**
The Bruininks-Oseretsky Test of Motor Proficiency (BOT)
Physical and Neurological Evaluation for Soft Signs (PANESS)
Hand Strength Dynamometer
Domain 2: Cognitive function and Language
Differential Ability Scales – 2nd edition (DAS-II)
Domain 3: Working memory/Executive function/Attention
Digit Span Backward
Verbal Fluencies: (A Developmental Neuropsychological Assessment, NEPSY)
Conners' Continuous Performance Test (CPT-II) (5–18+ yrs)/Kiddie CPT_(4–5 yrs)
Domain 4: Self-image and social function
Parent Questionnaires (filled out by mother in all cases except 2)
The Child Behavior Checklist (CBCL)
Child Self-Report Questionnaires (completed by the child)
Self-Concept. The Piers-Harris Self Concept Scale (SCS), Second Edition (ages 7–18 years).
Depression. The Children's Depression Inventory (CDI)

Table 2:

Baseline Demographic and IQ Information (Mean ± SD)

	KS-Ox	KS-Pl	P-value*
N	43	41	
Chronologic age	6.9±2.2	8.3±2.7	0.01
Socioeconomic status	52±10	53±9	0.55
% Caucasian	72%	76%	0.92
% 47,XXY	95%	95%	0.99
DAS Verbal cluster	95±12	95±16	0.67
DAS Nonverbal cluster	98±14	99±13	0.89

* t-test or Fisher Exact test

Table 3:

Longitudinal primary and secondary outcome analysis results (t-scores, Standard Scores, Mean±SEM)

	KS-Ox			KS-Pl			P-value*
	BL^	12 months	24 months	BL	12 months	24 months	24 months
Primary Outcome analysis Domain 1: Motor function/Strength							
BOT (N) SS~	**35**	**35**	**35**	**36**	**36**	**36**	
BOT Upper limb speed	86±2	90±2	92±1	87±2	88±2	89±2	0.17
BOT Strength	91±2	89±2	88±1	87±2	86±2	85±1	0.17
BOT Visual-motor control	89±3	85±1	86±1	86±3	84±1	81 ±1	**0.005**
Hand Dynamometer (N) SS	**34**	**34**	**34**	**36**	**36**	**36**	
Mean SS~Dominant hand	116±2	119±2	123±2	114±3	118±2	118±2	0.06
PANESS SS	**21**	**21**	**21**	**24**	**24**	**24**	
PANESS Dominant hand	80±5	82±4	91±3	83±5	82±4	91±3	0.87
Domain 2: Cognitive function, Verbal							
DAS SS~(N)	**35**	**35**	**35**	**36**	**36**	**36**	
General Conceptual Ability	95±2	96±2	96±2	95±2	94±2	94±2	0.44
Verbal Cluster	94±2	95±2	92±2	97±3	93±2	90±2	0.57
Nonverbal Cluster	98±2	101±2	102±2	99±2	98±2	101±2	0.55
Spatial Cluster (N)	94±3 (22)	97±2(22)	96±2(22)	90±3(24)	93±2(24)	94±2(24)	0.65
Domain 3: Working memory/Executive function/Attention							
Digit Span SS~(N)	**29**	**29**	**29**	**33**	**33**	**33**	
Digit Span Backward	96±3	92±3	96±2	94±3	92±2	92±2	0.23
Fluencies SS~	**12**	**12**	**12**	**17**	**17**	**17**	
Phonetic fluency	90±4	93±3	95±3	94±4	90±3	91±2	0.36
Semantic fluency	97±3	105±4	99±4	104±5	98±3	94±3	0.37
CPT SS~(N)	**32**	**32**	**32**	**31**	**31**	**31**	
Omissions	82±5	84±4	88±4	88±4	80±4	83±4	0.41
Commissions	97±2	100±3	101±2	95±3	98±3	100±2	0.73
Hit React Time	89±3	87±4	84±3	90±3	88±4	80±3	0.40
Variability	87±2	85±2	86±2	85±2	84±2	86±2	0.95
Perseverations	80±5	72±5	76±5	72±6	71±5	74±5	0.78
Domain 4 Social Function							
CBCL t-scores^^(N)	**35**	**35**	**35**	**36**	**36**	**36**	
Behavior Total	58±2	57±1	56±1	60±2	59±1	59±1	0.16
Internalizing Total	58±2	55±1	54±1	57±2	58±1	57±1	0.10
Externalizing Total	51±2	52±1	51±1	55±2	53±1	54±1	0.24
Withdrawn	60±2	56±1	56±1	57±1	57±1	57±1	0.34

	KS-Ox			KS-Pl			P-value*
	BL^	12 months	24 months	BL	12 months	24 months	24 months
Somatic complaints	60±2	58±1	56±1	59±1	59±1	59±1	0.10
Anxious/ depressed	58±2	56±1	55±1	58±2	59±1	59±1	**0.03**
Social problems	60±2	58±1	59±1	62±2	61±1	64±1	**0.01**
Thought problems	59±2	56±1	56±1	56±1	57±1	56±1	0.81
Attention problems	63±2	61±1	60±2	62±2	65±1	63±2	0.09
Delinquent behavior	55±1	56±1	55±1	56±1	56±1	55±1	0.69
Aggressive behavior	55±1	57±1	55±1	58±2	56±1	57±1	0.31
Sex problems	55±1	55±1	53±1	55±1	53±1	52±1	0.52
Piers Harris SS~ (N)	**16**	**16**	**16**	**20**	**20**	**20**	
Behavioral Adjustment	101±4	104±3	104±3	102±3	103±2	102±3	0.71
Intellectual/School Status	99±4	100±2	99±3	101±3	100±2	97±2	0.51
Physical Appearance	103±3	103±3	103±3	105±2	105±2	105±2	0.56
Freedom from Anxiety	97±4	105±3	106±2	102±3	102±2	99±2	**0.04**
Popularity	94±4	98±3	100±3	97±3	98±2	97±3	0.55
Happiness/Satisfaction	103±3	106±2	107±3	107±2	105±2	103±2	0.27
Total	99±5	104±3	105±3	102±3	103±2	100±2	0.15
CDI t-scores^^ (N)	**15**	**15**	**15**	**21**	**21**	**21**	
Total	47±3	46±1	45±2	48±2	48±1	47±2	0.50
Negative mood	46±2	46±2	46±2	48±2	46±1	47±2	0.60
Interpersonal problems	52±3	48±2	45±2	48±2	50±1	50±1	**0.02**
Ineffectiveness	47±2	47±2	46±2	49±2	49±2	50±2	0.13
Anhedonia	48±3	50±2	51±2	51±2	50±2	50±2	0.74
Negative self esteem	46±2	43±1	41±1	46±1	45±1	44±1	0.08

*ANCOVA LSM±SE for change from baseline at 2 years, adjusted for differences in baseline value, age, SES

^unadjusted baseline values mean±SEM

^^Scores are expressed as t-scores with mean of 50 and SD of 10. Lower scores imply better function and higher scores indicate more problem behaviors. For the CBCL, the 3 social competency scales are scored such that higher scores indicate better social competence.

~Scores are expressed as standard scores with mean of 100 and standard deviation (SD) of 15. Higher scores imply better function.