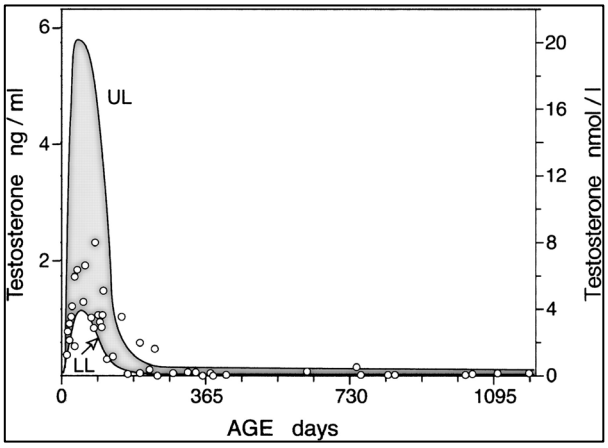
Testosterone in Infants with XXY

Clinical Trials Design Final Paper
Nicole Tartaglia, Shideh Majidi, Shanlee Davis

I. Background and Scientific Setting

Klinefelter syndrome (KS) is a common genetic disorder in which males have an extra X chromosome (47,XXY) and is associated with motor and psychological impairments that begin in childhood, primary gonadal failure in puberty, and disorders of insulin resistance in adulthood. With a prevalence of 1 out of every 450-650 males, there are assumed to be over 62,000 children in the United States with KS, although it is currently underdiagnosed [1, 2]. Non-invasive prenatal testing (NIPT) is quickly becoming widely available and can reliably identify sex chromosome aneuploidies [3]. This test may soon be offered to all pregnant women as first line screening regardless of age or other risk factors, increasing the prenatal diagnosis rate for KS by five to ten-fold. With this change in the landscape, there will be a greater demand for evidence-based research in the natural history, prevention, and treatment of complications and comorbidities in KS, particularly in infancy and early childhood.

Evidence for early hypogonadism in KS. Infants with KS are known to have lower muscle tone and a higher incidence of underdeveloped genitalia in infancy, which suggests that androgen deficiency begins early in life [4, 5]. Normal male infants have an activation of their hypothalamic-pituitary-gonadal axis with a surge of LH, FSH, and testosterone at 2-3 months of age, referred to as the "mini-puberty of infancy." The mini-puberty occurs in infants with KS, however there is evidence the peak testosterone levels are lower than average (see figure) [5-7]. Following the infant surge, testosterone levels in males with KS are usually normal until mid-puberty, when hormone concentrations become characteristic of hypergonadotropic hypogonadism [8-10]. Therefore, as there is evidence to support androgen insufficiency in infants with KS, the normal mini-puberty period represents a biologically promising target for testosterone therapy. This treatment is currently given to males with micropenis with a widely accepted protocol and no significant safety concerns [11]. As around 5% of infants with KS have micropenis and more than a third have poor penile growth, infants with KS often receive testosterone treatment for this indication [4, 7]. At some institutions, this is considered the standard of care for all infants with KS, despite a lack of scientific evidence to support this practice [12]. The only study investigating testosterone treatment in KS infants is a retrospective observational study published in 2013 that identified neurodevelopmental benefit for KS boys with a history of testosterone treatment [13]. *A gap currently exists in the potential of short and long-term outcomes of*



testosterone treatment in infants with KS, and this study will explore body composition and motor development as short term outcome measures.

Evidence for an unfavorable body composition and relationship to morbidity and mortality in KS. Adult males with KS have higher fat to lean body mass ratio and higher rates of metabolic syndrome, type 2 diabetes, and cardiovascular-related mortality [14-19]. These findings correlate with the degree of androgen deficiency and are hypothesized to be secondary to hypogonadism [16, 17]. Despite this, there are no randomized controlled trials evaluating the efficacy of testosterone in reversing unfavorable body composition, metabolic syndrome, or other precursors of cardiovascular disease that are already present. One cross-sectional study has shown a trend toward lower total body fat, cholesterol, and fasting insulin levels in those on testosterone treatment, although these findings were not significant [16]. Recent studies suggest that current approaches to intervention may be too late, as increased body fat mass, percent body fat, waist circumference, insulin resistance, and unfavorable lipid profiles are present prior to puberty [20, 21]. However, markers of insulin resistance have never been studied in infants and young children with KS. Body composition is a particularly pertinent surrogate marker of insulin resistance as increased fat to lean muscle mass may also contribute to motor development, strength, and endurance in infancy and childhood. We have found children with KS have a high prevalence of hypotonia (65%), motor delays (53%), poor coordination (66%) and low endurance (47%) (unpublished, Tartaglia PI). Body composition is also directly affected by testosterone, and testosterone treatment has been shown to favorably alter body composition in males with hypogonadism[22]. There have been no studies evaluating the effects of testosterone on body composition or motor development in infants with KS. *This study aims to address the question of whether exogenous testosterone during the expected mini-puberty period of infancy in boys with KS has beneficial short-term effects on body composition and development.*

Specific Aim and hypotheses:

Specific Aim: To determine if a short course of exogenous testosterone (T) during the mini-puberty period of infancy has a favorable effect in male infants with KS on body composition, muscle tone, motor development and penile length.

Primary Hypothesis: T treatment will result in a decrease in body fat percentage as measured by the PEA POD

Secondary Hypotheses: T treatment will increase muscle tone, motor development, and penile length compared to those not treated.

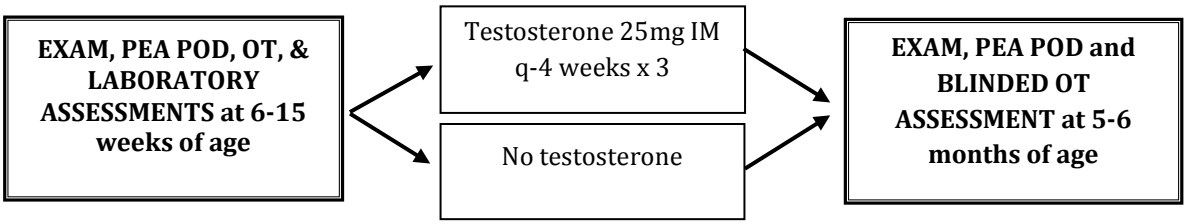
II. Synopsis of Proposed Study

Study Design. Randomized controlled trial of testosterone therapy versus no treatment in infants with Klinefelter syndrome/XXY.
Type of Trial. Superiority of a less-than hypothesis.
Study population.

Inclusion criteria: Male infants 6-15 weeks of age with 47,XXY karyotype.

Exclusion criteria: Gestational age at birth <36 weeks, birth weight <5%ile or >95% for gestational age, history of thrombosis in a first degree relative, and exposure to androgen therapy outside of the study protocol.

Study treatments. All subjects will be randomized upon enrollment to 1) Testosterone cypionate (200 mg/mL) 25 mg given via intramuscular injections every four weeks for three doses or 2) no pharmacologic intervention.



Study measurements. Assessments will be done at baseline and three months later. For body fat percentage and anthropometric measurements, age-appropriate z-scores will be calculated based on published normative data [23]. Standard scales will be calculated for the developmental assessments [24, 25].

Table 1. Study Measurements: Subjects will have an initial study visit (V1) at 6-15 weeks of life and another (V2) at 5-6 months of life. Investigators will remain blinded (B) to the intervention group as indicated.

Outcome	**V1**	**V2**	**B**	**Description/Method**
Body composition *Primary outcome*	X	X	X	The PEA POD (air-displacement plethysmograph) will non-invasively quantify fat and non-fat mass as well as %BF in infants.
Muscle tone and motor development	X	X	X	An occupational therapist (OT) will administer validated assessments: Alberta Infant Motor Scale and Peabody Motor Scales.
Gonadal Function	X		X	LH, FSH, TT, INHB, and AMH will be analyzed in the lab of Dr. Lahlou. These values will be compared to age-based laboratory normative data.
Physical Exam	X	X	*	Complete physical exam including anthropometric measurements. *Investigator assessing penile length will be blinded.

Statistical design.

Overall Design. This is a randomized controlled trial.

Endpoints. The primary endpoint is the change in percent body fat z-score from V1 to V2. Secondary endpoints include the change in standard scores on the validated OT assessments from V1 to V2 and change in stretched penile length. Exploratory outcomes include serum gonadal function biomarkers measured at baseline and the safety profile.

Analysis plan. The mean difference of the change in percent body fat z-score from V1 to V2 (primary endpoint) will be compared between the treated and untreated groups using a two sample t-test. The investigators have determined a mean difference of -1 standard deviation between the treated and untreated group would be clinically significant. Based on preliminary data, the standard deviation in the primary endpoint (change in body fat percentage z-scores) is 1.05.

Sample size calculation. For 90% power to detect a mean difference of -1 assuming an alpha of 0.05 and a standard deviation of 1.05, we would need 24 subjects per group. To allow for up to ~12% drop-out and/or missing data, we will aim to enroll a total of 54 subjects.

III. Study Implementation and Conduct

The Setting. Children's Hospital Colorado is home of the eXtraordinarY Kids Clinic, a nationally unique multidisciplinary clinic for clinical care and research for children with sex chromosome aneuploidies like KS. This clinic has had over 600 visits for boys with KS and has served as an excellent referral base for clinical research. The investigative team has experts in developmental pediatrics, andrology, neonatal body composition, insulin resistance syndromes, genetics, psychology, and physical, occupational, and speech therapy.

Recruitment and retention plan. Participants will be recruited nationally through the eXtraordinarY Kids Clinic, AXYS parent support group, and outreach to genetic counselors. A pilot study conducted at our institution the past several months has proven feasibility in recruiting infants with KS in the mini-puberty age range to participate in an interventional trial, with enrollment exceeding recruitment goals. Recruitment success is largely due to strong relationships with the national parent support group, AXYS, as well as genetic counselors who advise parents receiving a prenatal diagnosis of 47,XXY. In addition, to support both recruitment and retention, study participation will guarantee access to the eXtraordinarY Kids Clinic, where there is currently a year-long waitlist. A clinic visit will be coordinated with the final study visit.

Randomization procedures. Completely randomized design will be used to randomize subjects. A randomization table will be used—an Excel spreadsheet will be used with random numbers determined for each subject. If the random number is less than 0.5, then the subject will receive T. Otherwise, they will receive placebo. The randomization table will be kept with

the pharmacist so the PI and subject/family will not know what group the subject has been randomized in until the end of Visit 1.

Procedures for blinding. For ethical and logistical reasons, the PI and parents will be aware of the intervention group assigned, however other study team members will remain blinded to the randomization assignment. To maintain blinding of the team members, visit encounters will be created in the electronic medical record for dates testosterone injections would be due to be received for all participants, including subjects randomized to no treatment. Research visits will be password protected in the electronic medical record and blinded study team members will not have to access these encounters. Parents will be instructed not to disclose the randomization assignment to any study team members.

Procedures for minimizing missing data. The study aims to enroll 54 subjects, which allows for up to ~12% drop-out and/or missing data. To minimize missing data, data collection is limited to the lowest needed to obtain all necessary information (2 visits). The informed consent documents will emphasize the importance of collecting the outcome data even in those who choose to discontinue treatment. Based on our pilot trial experience, the primary threat to retention will be subjects who are randomized to no treatment. To minimize this risk, subjects randomized to no treatment will have the opportunity to receive testosterone after completion of the study protocol.

Human subjects research. The PI holds an Investigational New Drug (IND) with the FDA to study testosterone cypionate in infants with KS. The study will seek approval from the local IRB and additional local regulatory bodies who oversee human subjects research.

REFERENCES

1. Bojesen, A., S. Juul, and C.H. Gravholt, *Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study.* J Clin Endocrinol Metab, 2003. **88**(2): p. 622-6.
2. Herlihy, A.S., et al., *The prevalence and diagnosis rates of Klinefelter syndrome: an Australian comparison.* Med J Aust, 2011. **194**(1): p. 24-8.
3. Lo, J.O., et al., *Noninvasive prenatal testing.* Obstet Gynecol Surv, 2014. **69**(2): p. 89-99.
4. Ratcliffe, S.G., G.E. Butler, and M. Jones, *Edinburgh study of growth and development of children with sex chromosome abnormalities. IV.* Birth Defects Orig Artic Ser, 1990. **26**(4): p. 1-44.
5. Lahlou, N., et al., *Clinical and hormonal status of infants with nonmosaic XXY karyotype.* Acta Paediatr, 2011. **100**(6): p. 824-9.
6. Lahlou, N., et al., *Inhibin B and anti-Mullerian hormone, but not testosterone levels, are normal in infants with nonmosaic Klinefelter syndrome.* J Clin Endocrinol Metab, 2004. **89**(4): p. 1864-8.
7. Ross, J.L., et al., *Early androgen deficiency in infants and young boys with 47,XXY Klinefelter syndrome.* Horm Res, 2005. **64**(1): p. 39-45.
8. Aksglaede, L., et al., *Clinical and biological parameters in 166 boys, adolescents and adults with nonmosaic Klinefelter syndrome: a Copenhagen experience.* Acta Paediatr, 2011. **100**(6): p. 793-806.
9. Salbenblatt, J.A., et al., *Pituitary-gonadal function in Klinefelter syndrome before and during puberty.* Pediatr Res, 1985. **19**(1): p. 82-6.

10. Wikstrom, A.M., et al., *Are adolescent boys with Klinefelter syndrome androgen deficient? A longitudinal study of Finnish 47,XXY boys.* Pediatr Res, 2006. **59**(6): p. 854-9.
11. Carrillo, A., *Disorders of Sexual Differentiation*, in *Pediatric Endocrinology*, Lifshitz, Editor. 2007, Informa Healthcare USA: New York, NY.
12. Wosnitzer, M.S. and D.A. Paduch, *Endocrinological issues and hormonal manipulation in children and men with Klinefelter syndrome.* Am J Med Genet C Semin Med Genet, 2013. **163C**(1): p. 16-26.
13. Samango-Sprouse, C.A., 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.* Am J Med Genet A, 2013. **161A**(3): p. 501-8.
14. Andersen, N.H., et al., *Left ventricular dysfunction in Klinefelter syndrome is associated to insulin resistance, abdominal adiposity and hypogonadism.* Clin Endocrinol (Oxf), 2008. **69**(5): p. 785-91.
15. Bojesen, A., C. Host, and C.H. Gravholt, *Klinefelter's syndrome, type 2 diabetes and the metabolic syndrome: the impact of body composition.* Mol Hum Reprod, 2010. **16**(6): p. 396-401.
16. Bojesen, A., et al., *The metabolic syndrome is frequent in Klinefelter's syndrome and is associated with abdominal obesity and hypogonadism.* Diabetes Care, 2006. **29**(7): p. 1591-8.
17. Gravholt, C.H., et al., *Body composition, metabolic syndrome and type 2 diabetes in Klinefelter syndrome.* Acta Paediatr, 2011. **100**(6): p. 871-7.
18. Jiang-Feng, M., et al., *Prevalence and risk factors of diabetes in patients with Klinefelter syndrome: a longitudinal observational study.* Fertil Steril, 2012. **98**(5): p. 1331-5.
19. Swerdlow, A.J., et al., *Mortality in patients with Klinefelter syndrome in Britain: a cohort study.* J Clin Endocrinol Metab, 2005. **90**(12): p. 6516-22.
20. Bardsley, M.Z., et al., *Insulin resistance and metabolic syndrome in prepubertal boys with Klinefelter syndrome.* Acta Paediatr, 2011. **100**(6): p. 866-70.
21. Aksglaede, L., et al., *Normal bone mineral content but unfavourable muscle/fat ratio in Klinefelter syndrome.* Arch Dis Child, 2008. **93**(1): p. 30-4.
22. Blouin, K., A. Boivin, and A. Tchernof, *Androgens and body fat distribution.* J Steroid Biochem Mol Biol, 2008. **108**(3-5): p. 272-80.
23. Fields, D.A., et al., *Longitudinal body composition data in exclusively breast-fed infants: a multicenter study.* Obesity (Silver Spring), 2011. **19**(9): p. 1887-91.
24. Snyder, P., et al., *Concurrent validity and reliability of the Alberta Infant Motor Scale in infants at dual risk for motor delays.* Phys Occup Ther Pediatr, 2008. **28**(3): p. 267-82.
25. Provost, B., et al., *Concurrent validity of the Bayley Scales of Infant Development II Motor Scale and the Peabody Developmental Motor Scales-2 in children with developmental delays.* Pediatr Phys Ther, 2004. **16**(3): p. 149-56.