COMIRB Protocol

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Protocol #: 14-1720
Project Title: Body Composition in Infants with Klinefelter Syndrome and Effects of Testosterone Treatment

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Version Date: 3/9/2015

I. Hypotheses and Specific Aims:

Klinefelter syndrome (KS) affects more than 62,000 children in the United States and is associated with motor and psychological impairments in childhood, primary gonadal failure in puberty, and metabolic syndrome and related disorders in adolescence and adulthood (1). Older children and adolescents with KS have been shown to have an unfavorable body composition, with a greater adipose tissue to lean muscle ratio that may be associated with the increased risk of metabolic syndrome, type 2 diabetes, and cardiovascular-related mortality in adults with KS (2-4). Research suggests these conditions are related to hypogonadism and preventable with testosterone treatment, although there have been no randomized controlled trials assessing efficacy in reversing unfavorable body composition once established (5). Several observations indicate that infants with KS have androgen insufficiency, specifically a higher incidence of underdeveloped genitalia, lower muscle tone, and lower peak testosterone levels during the infant hormone surge known as the "mini-puberty." Furthermore, testosterone is often used off-label in infants with KS in the absence of an evidence base (6). We hypothesize that body composition in infants with KS is abnormal and associated with lower testosterone levels and, furthermore, can be improved with supplemental testosterone therapy during the normal testosterone surge of infancy. To test this hypothesis, we will undertake the following specific aims.

1. **Specific Aim 1: To assess body composition and gonadal function in infants with KS.** We will perform air displacement plethysmography (PEA POD) and serum hormone concentrations during the mini-puberty period in 20 infant males with a prenatal diagnosis of KS (47,XXY) and compare the results to published normal values for male infants of matched age.
    a. Hypothesis 1a: Infants with KS, like older males with KS, will have a higher body fat percentage when compared to published normal values.
    b. Hypothesis 1b: Infants with KS will have lower serum testosterone levels compared to published normal values.
    c. Hypothesis 1c: Serum testosterone levels will inversely correlate with body fat percentage, similar to post-pubertal males with KS.
2. **Specific Aim 2: To determine if a short course of testosterone therapy during the mini-puberty period improves body composition in infants with KS**. We will randomize half of the infants to receive intramuscular testosterone at doses commonly used for treatment of underdeveloped genitalia in this population and assess body composition as the primary outcome measure. We will compare both treated and untreated participants with matched controls from a large local population database. Secondary outcome measures will include penile length, muscle tone, and motor development.
    a. Hypothesis 2a: Testosterone treatment will have a favorable effect on body composition, penile length, muscle tone, and motor development compared to those not treated.
    b. Hypothesis 2b: Untreated KS males will have a higher body fat percentage than matched controls from the local population database while treated KS males will not differ from matched controls.

II. Background and Significance:

Klinefelter syndrome (KS) is a common genetic disorder in which males have an extra X chromosome (47,XXY). 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 (7,8). Non-invasive prenatal testing (NIPT) is quickly becoming widely available and can reliably identify sex chromosome aneuploidies (9). 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. Expectant parents are faced with decisions, including termination of affected fetuses, with very limited evidence-based guidance since the syndrome is understudied, particularly in children with a prenatal diagnosis. Likewise, pediatric healthcare providers have few guidelines for managing these children and patterns of care vary greatly (1). *Thus, at a time when prenatal diagnosis of KS will most likely greatly increase, a gap exists in our understanding of the natural history, prevention, and treatment of complications and comorbidities in KS, particularly in infancy and early childhood*.

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 (4,5,10-13). These findings correlate with the degree of androgen deficiency and are hypothesized to be secondary to hypogonadism (11,12). 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 (11). 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 (2,3). *No studies have looked at body composition in infants or toddlers with KS. Thus a gap exists in determining when increased body fat and decreased muscle mass develop. This study would start to address this critical knowledge deficit.*

If hypogonadism is the underlying pathophysiology of an unfavorable body composition in KS and this finding is present in pre-pubertal boys, testosterone insufficiency must be present prior to pubertal activation of the hypothalamic-pituitary-gonadal axis. 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 (14,15). 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 (15-17). Following the infant surge, testosterone levels in males with KS are usually normal until mid-puberty, when hormone concentrations become characteristic of hypergonadotropic hypogonadism (18-20). 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 (21). 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 (14,17). At some institutions, this is considered the standard of care for <u>all</u> infants with KS, despite a lack of scientific evidence to support this practice (22). 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 (23). *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.*

Body composition is affected by testosterone, with inverse correlations between serum testosterone levels and percent body fat in multiple populations including adult men with KS and normal neonates. Testosterone treatment has been shown to favorably alter body composition in males with hypogonadotropic hypogonadism (24). Body composition is a clinically meaningful outcome measure of testosterone treatment, as unfavorable body composition may have direct implications in infancy and childhood, contributing to the motor delays and decreased strength, endurance, and athletic ability seen in KS. Furthermore, an unfavorable body composition profile in infancy may be a surrogate marker for an altered metabolism contributing to adult diseases, such as the metabolic syndrome, fatty liver disease and type 2 diabetes (12). *This study aims to address the question of whether body composition in infants with KS is a measurable and modifiable outcome of testosterone during the expected mini-puberty of infancy.*

III. **Preliminary Studies/Progress Report:**

<u>Previous experience with this population.</u> Early in her career the PI worked as a research assistant with Dr. Nicole Tartaglia (co-investigator) collecting data and interacting with hundreds of patients with various sex chromosome aneuploidies and their families. Select descriptive data from this work derived from parent report and physical exams of children with KS includes a high prevalence of hypotonia (65%), poor coordination (66%), and low endurance (47%).

<u>Children's Hospital Colorado eXtraordinarY Kids Clinic</u>. The eXtraordinarY Kids Clinic is a multidisciplinary clinic, nationally unique to CHCO, that provides comprehensive clinical care for children with X & Y chromosome

disorders. Dr. Nicole Tartaglia (co-investigator) is the Medical Director of the clinic and has an extensive publication record in this population. Dr. Phil Zeitler (co-investigator) is the primary endocrinologist for the clinic and has expertise in the endocrinological manifestations in KS, as well as extensive research in the areas of body composition, insulin resistance, and obesity-related comorbidities in children. The clinic has been an excellent base for recruitment into clinical-translational research with over 950 patient visits (~65% with KS). Due to the nationally-recognized expertise in KS, the clinic's genetic counselor receives over 25 calls annually from expectant parents seeking more information and research opportunities. Descriptive data from boys with KS < 5 years of age participating in an ongoing observational study revealed 88% had hypotonia and 62% had delays in motor development. More strikingly, 38% had received testosterone injections as infants despite the lack of evidence to support this practice.

Local expertise in infant body composition. Several active studies on campus currently utilize the PEA POD for measurement of body composition in infants. Collaboration with researchers who have expertise in this field, including Regina Reynolds, MD (co-investigator), has been important in development of this protocol. Additionally, Dana Dabelea, PhD, has offered a de-identified data set for a large population-based healthy cohort of 5 month olds (Healthy Start Study) to serve as a resource for matched normal values. These local resources make this the ideal setting for a successful study of body composition in KS infants.

Other relationships to help with recruitment. Perinatal genetic counselors at University Hospital and Kaiser have agreed to assist with identifying potential study subjects with a prenatal diagnosis of KS. In addition, AXYS, the parent support group for all sex chromosome aneuploidies with over 7,800 contacts, fully supports of recruitment for this study.

IV. Research Methods

A. Outcome Measure(s):

The primary outcome measure will be body composition (percent fat mass) as determined by the PEA POD. Secondary outcome measures will be 1) pre-treatment serum and urine gonadotropins and serum testosterone levels, 2) assessment of tone and motor abilities by an occupational therapist blinded to treatment assignment, and 3) stretched penile length.

B. Description of Population to be Enrolled:

Twenty male infants with KS (karyotype 47,XXY) will be recruited to participate in this pilot study. The age of the infants was chosen 1) to allow assessment of gonadal status during the expected mini-puberty period of infancy, 2) to allow the final assessment to be completed before the infant is 10kg, as this is the upper weight limit for the PEA POD.

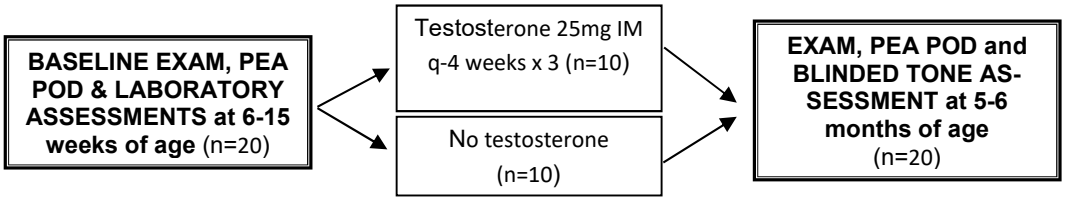
Inclusion criteria: Male infants 6-15 weeks of age with 47,XXY karyotype.

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.

Subjects will be recruited through (1) the eXtraordinarY Kids Clinic at Children's Hospital Colorado, (2) Obstetrics and genetic counseling departments at University Hospital and other perinatal centers in the Denver area, and (3) Outreach from KS-related support groups including postings on websites and in newsletters.

C. Study Design and Research Methods

Overall study design. This is a pilot study of body composition and testicular function in infants with KS compared to published norms for males by age (Specific Aim 1), followed by a randomized trial of a short course of testosterone therapy with evaluation of change in body composition and motor development (Specific Aim 2). Total study duration for each individual subject is 10-12 weeks.



Study Timeline and Procedures.

Week	Activity	Subjects	Personnel	Location
1	Baseline study visit • Consent • Medical History • Physical Examination • OT evaluation • PEA POD assessment • Blood and urine collection • Randomization	All	 PI or RA PI PI Blinded OT PI/Co-investigator CTRC RN Research Team	Pediatric CTRC
1	Testosterone injection #1	Treatment only		Pediatric CTRC
2	Phone call to parents	All	PI or RA	N/A
4	Testosterone injection #2	Treatment only		Pediatric CTRC
5	Phone call to parents	All	PI or RA	N/A
8	Testosterone injection #3	Treatment only		Pediatric CTRC
10-12	Final study visit • Medical History • Physical Examination • OT evaluation • PEA POD assessment	All	 PI Blinded Peds Endo Blinded OT PI/Co-investigator	Pediatric CTRC

Screening. Potential subjects will be screened for inclusion and exclusion criteria via phone conversation prior to the first study visit. The consent document will be provided to the parents and discussed with a member of the research team. If the subject qualifies and parents are interested in participating in this research study, the initial study visit will be scheduled between 6 and 15 weeks of age.

Baseline study visit: This initial visit will occur at 6-15 weeks of age and will take approximately 3 hours.

Randomization. At the conclusion of the baseline study visit, subjects will be randomized to 1) Testosterone treatment or 2) no pharmacologic intervention. A large opaque manila envelope will contain smaller sealed envelopes each of which hold a single piece of paper with the letter “T” (testosterone treatment) or “X” (no treatment) written on them. We will randomize in blocks of 10. A member of the research team will pull out a single envelope that will contain the group to which the subject will be assigned. This study will not be blinded or include a placebo intervention due to ethical limitations of giving placebo injections. However, all outcomes that are not entirely objective (physical exam, OT assessment) will be performed by a study staff member who is blinded to the participants treatment assignment. The parents of participants will be counselled not to disclose their randomization status to blinded study personnel.

Injection visits (treatment group only.) These will be arranged to be done at the outpatient CTRC at CHCO. If it is not feasible for the subject to return to CHCO for injections #2 and #3, the parents will be given the option to do the injection at home. The parent will be taught by a medical professional on the study team how to draw up and administer the intramuscular injection. Teaching families to administer home injections is standard practice for clinical care. Given the highly motivated population being enrolled in this study, it is reasonable to provide this option for families who live a long distance from Children’s Hospital Colorado.

Phone calls to parents: This will serve to assess for adverse events as well as facilitate subject retention.

Final study visit. The final study visit will occur 10-12 weeks after the baseline study visit (2-4 weeks after receiving the final testosterone injection for those in the treatment group). Estimated time for this visit is approximately 3 hours. At that time, subjects will be offered the opportunity to be seen clinically (outside of the research protocol) in the eXtraordinarY Kids clinic for a full developmental assessment.

Patient participation: Parents/patients may choose to stop participation at any point in the study. Mandatory patient discontinuation criteria will include any serious adverse event related to the study protocol including but not limited to thrombosis, hemorrhagic event, and anaphylaxis.

D. Description, Risks and Justification of Procedures and Data Collection Tools:

Medical history. Maternal and infant medical history, medications, therapies or other interventions, and infant feeding method will be documented. No anticipated risk to subject. See data collection sheet.

Physical examination. Length and weight will be obtained by CTRC nurses. A comprehensive physical exam will be performed by a pediatric endocrinologist blinded to the treatment arms. Stretched penile length, tanner staging, and testicular size by Prader orchiometer will be done by a pediatric endocrinologist blinded to the treatment arms. No anticipated risk to subject.

PEA POD. The PEA POD provides a reliable, non-invasive, quantitative assessment of fat mass and non-fat mass utilizing air-displacement plethysmograph technology in infants weighing less than 10 kilograms (25). This instrument has been validated with other methods of assessing body composition, such as deuterium dilution and dual-energy X-ray absorptiometry, and normative values for healthy, term, average-gestational age males are published from birth through 6 months (26). The PEA POD is safe and comfortable for infants and takes less than 10 minutes to perform. This body composition assessment method was chosen for its low risk to the patient compared to more intensive and occasionally invasive measures of body composition. This assessment will be performed by the PI with a blinded study investigator (Dr. Reynolds and/or CTRC RN).

Alberta Infant Motor Scale (AIMS). The AIMS is a performance-based and norm-referenced measure of infant gross motor maturation from birth to 18 months. It is intended to evaluate gross motor development over repeated assessments and has good reliability and predictive validity for later gross motor development (27,28). In a systematic review of neurodevelopmental tests, the AIMS had the best psychometric properties and clinical utility (29). The assessment will be administered by an OT (co-investigator) at the baseline and the final visit. The OT will remain blinded to the treatment arms. There is no anticipated risk to with this assessment.

Peabody Developmental Motor Scales 2 (PDMS-2). This is a standardized assessment tool for infant development and includes six subtests to assess gross and fine motor scales from birth to 5 years. It is one of the most common tools for discriminating normal from abnormal infant motor development (30). This instrument was chosen as it is more broad assessment of motor development compared to the AIMS, has normative reference data, and strongly correlates with other motor assessments. This will be administered by an OT (co-investigator) at both the baseline and the final visit. The OT will remain blinded to the treatment arms. . Change in raw scores will be evaluated in both treatment and non-treatment groups. There are no anticipated risks to the subject with this assessment.

Movement Assessment of Infants (MAI). This standardized instrument quantifies motor behaviors and contains four different domains: tone, primitive reflexes, automatic reactions, and volitional movement (31). We will be using the first domain only (tone) for the objective assessment of muscle tone. This will be administered by an OT (co-investigator) at both the baseline and the final visit. The OT will remain blinded to the treatment arms. There are no anticipated risks to the subject with this assessment.

Laboratory assessments. Venipuncture will be performed at the initial study visit for measurement of luteinizing hormone (LH), follicle stimulating hormone (FSH), total testosterone (TT), anti-mullerian hormone (AMH), inhibin B, and leptin. The serum will be spun, frozen, and saved in Endocrine Departmental freezers. These samples will be sent to the lab of collaborator, Dr. Najiba Lahlou in Paris, France for analysis of all the above biomarkers. Dr. Lahlou has a special interest in sex chromosome aneuploidy and has published one of the few studies examining gonadotropins and markers of testicular function during the mini-puberty period in KS (16). Urine collection bag will be placed for measurement of urine gonadotropins in the lab of Nanette Santoro, MD in the Department of Obstetrics and Gynecology at the University of Colorado. Laboratory assessments will be obtained in the morning if possible.

Testosterone treatment. Subjects randomized to testosterone treatment will receive testosterone cypionate (200 mg/mL) 25 mg given via intramuscular injection every 4 weeks for three doses. This treatment dosing and dura-

tion is accepted practice for treatment of micropenis in this age group without reported side effects (21). The potential minor side effects of testosterone therapy include inflammation and/or pain at the site of injection, hypersensitivity reactions, acne, and adrenarche. Serious side effects are not anticipated, however androgen therapy at higher doses and/or for longer duration has been associated with venous thromboembolus, water retention, and priapism. Given the short duration and low dose of therapy, no routine laboratory screening will be done. Families of subjects will be contacted one week following the initial assessment (and first dose of testosterone for those being treated) to assess for any concerns the family may have.

E. Potential Scientific Problems:

Recruitment of the study population within the age window specified will be the primary challenge. However, the research team is well-positioned with the nationally recognized, eXtraordinarY Kids Clinic and relationships with other referral sources. If recruitment goals are not being met after the first year, inclusion/exclusion criteria and recruitment efforts will be evaluated. Other potential scientific problems include the many contributing factors influencing body composition. However, these will be minimized due to the young age of the children enrolled. We plan to exclude those born <36 weeks gestation and those born small or large for gestational age. We will collect data on factors that could potentially contribute to body composition, including maternal BMI, weight gain during pregnancy, maternal results on oral glucose tolerance testing, birth weight and gestational age, infant feeding method, and physical therapy interventions. We will control for any factors that are significantly different between the treatment and non-treatment groups. In addition, given introduction of solid foods alters body composition, we will provide parents with the current American Academy of Pediatrics recommendations for solely breastfeeding until 6 months of age.

F. Data Analysis Plan:

As a pilot study, feasibility will be assessed both qualitatively and quantitatively including enrollment and completion numbers, reasons for declining participation and/or withdrawing from participation, protocol challenges, and reasons for any data points that could not be collected.

For continuous outcomes, frequency distributions for each variable of interest at baseline and final study visit will be generated and examined for normality assumptions, and if not met, nonparametric equivalents will be used. Outliers and missing data will be evaluated on an individual basis, with checking of source documentation to ensure the value is correct. Due to the small study numbers, outliers will not be excluded and any missing data will be treated as missing without imputation.

Summary statistics for demographic variables, covariates known to affect body composition, hormone concentrations, and outcomes at baseline will be generated and reported as means and standard deviation (or nonparametric equivalent). For the primary outcome, change in percent body fat z-scores will be compared between groups using Welch's two-sided, two-tailed t-test with an a priori level of significance set at 5%. Secondary outcomes including percent fat mass, absolute measures of body composition parameters (fat mass, fat free mass, total mass), stretched penile length z-scores (and absolute penile lengths), other anthropometric measures, and motor skills scores will be analyzed in a similar fashion. As a secondary analysis, percent body fat at the final study visit will be compared between the treated group, untreated group, and data from normal local male controls at a median of 5 months of age using ANOVA followed by between group t-tests. No corrections for multiple comparisons are planned given the pilot nature of the study. All parent-reported adverse events and significant adverse events in the treatment group will be reported. No comparisons will be made between treated and untreated groups as these data are not being collected in the group not receiving treatment.

G. Summarize Knowledge to be Gained:

The results of this study will be important in 1) assessing the feasibility of recruitment and enrollment of this population into a larger clinical trial, and 2) determining the change in chosen outcome measures (body composition, motor development) following testosterone treatment to allow development of a statistical plan for a larger, multicenter study with both short and longer-term outcomes. The results of this study will impact clinical practice as currently up to 1/3 of infants with KS are treated with testosterone without existing research identifying the benefits or potential harm of the variable doses, durations, and administration methods. If testosterone therapy positively effects body composition and motor development in infants, this therapy may eventually be shown to prevent the insulin resistance and increased cardiovascular morbidity and mortality in this population. Furthermore, results of this work may be generalizable beyond KS in understanding the understudied relationship of hy-

pogonadism, body composition, and insulin resistance characteristic of obese boys, a problem of increasing significance secondary to the pediatric obesity epidemic.

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