Androgen Effects on Cognition in Klinefelter's Syndrome

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PROTOCOL: Androgen Effects on Cognition in Klinefelter's Syndrome, Control # 02F476, Judith Ross, M.D., Thomas Jefferson University

Specific Aims

Klinefelter's syndrome (KS) (1) is a relatively common genetic disorder that occurs in 1/500 males, is defined by the abnormal chromosome karyotype 47,XXY, and includes a characteristic physical, cognitive, and behavioral phenotype. The KS physical phenotype is based on testicular failure and tall stature. The KS cognitive phenotype includes language-based learning difficulties, dyslexia, impaired attention, working memory, and executive abilities, and diminished muscle tone, strength, and coordination. The KS behavioral phenotype involves poor self-image, shyness and increased social anxiety, starting in childhood.

The KS phenotype may be the result of androgen deficiency in utero, infancy and childhood. Androgen replacement is standard in adolescent and adult KS males but has not been used earlier in childhood. The goal of this randomized clinical trial is to study the effects of childhood androgen replacement on the KS neurocognitive and physical phenotypes. This randomized, placebo-controlled, longitudinal study tests a novel intervention in this population. We propose to replace androgen for two years in KS boys, between the ages of 4 and 12 years, using low, physiologic doses. We predict that multiple facets of the KS phenotype will improve with early androgen replacement: working memory, fine- and gross-motor coordination and speed, as well as self-esteem within the cognitive and behavioral phenotypes and muscle strength within the physical phenotype.

Klinefelter's syndrome is well suited for interventional studies because the disorder is relatively common in males, testicular failure is nearly universal, and the physical and cognitive phenotypic features have been characterized. Early androgen replacement in physiologic rather than pharmacologic dosages is a reasonable, appropriate, and safe consideration in this population. Therapeutic interventions for this relatively common disorder have not been forthcoming, and this represents a unique opportunity to replace a missing hormone and improve aspects of the cognitive and behavioral outcome. The primary specific aim of this study will test the hypothesis that androgen replacement in childhood is beneficial by comparing cognition and behavior in androgen-treated versus placebo KS groups. If successful, androgen replacement would commence early in childhood rather than adolescence or adulthood in Klinefelter's syndrome.

Specific Aim 1. Early androgen intervention, using a randomized clinical trial and treating for two years. We will evaluate five primary outcome variables: general cognition, language summary score, Working memory/executive function summary score, Motor function summary score, and Self-image and social function summary score.

Hypotheses regarding five primary outcomes:

1. Certain aspects of the KS physical, neurocognitive and behavioral phenotype will improve with childhood androgen replacement therapy.
    a. The working memory summary score will improve after two years in the androgen-treated versus the placebo group.

b. The motor function summary score will improve after two years in the androgen-treated versus the placebo group.
c. The self-image summary score will improve after two years in the androgen-treated versus the placebo group.

BACKGROUND AND SIGNIFICANCE

Klinefelter's syndrome (KS), first described in 1942 on the basis of testicular failure (1), occurs only in males, and is characterized by the abnormal chromosome karyotype 47,XXY (2). Unlike other chromosomal trisomies, 47,XXY does not frequently cause spontaneous abortuses, most likely related to inactivation of the supranumerary X-chromosome occurring in many tissues. The KS phenotype includes testicular failure, tall stature, and a characteristic cognitive and behavioral phenotype. Other KS-associated phenotypic findings include increased risk for osteoporosis, breast cancer, autoimmune thyroid disorders, and Type II diabetes mellitus (3). Most KS males were previously diagnosed in adolescence on the basis of testicular failure; however, increasing numbers are now detected through routine antenatal testing. Approximately 1 per 500-1000 males are affected (4). Mosaicism for a normal 46,XY line occurs in approximately 10-20% of KS cases and is associated with improved prognosis.

I. Testicular failure phenotype

The clinical spectrum for the testicular failure phenotype ranges from near complete androgen deficiency and gonadal failure starting prenatally to mild androgen deficiency with azoospermia in adulthood. Many KS boys have fetal onset of gonadal failure manifest by small testes and penis early in infancy or childhood and early symptoms of androgen deficiency. Others manifest testicular failure in adolescence or adulthood. The in utero testosterone deficiency would likely occur in the last trimester because testosterone deficiency earlier in gestation would result in ambiguity of the external genitalia with hypospadias, which is not characteristic of KS. Rather, a burst of testosterone synthesis in the last trimester stimulates penile growth and it is this later testosterone growth-stimulating effect on the genitalia that is missing in KS males.

A clue for diagnosis of KS earlier in infancy or childhood is the presence of small penis and testes, as exemplified by a set of twins discordant for KS (5). The testes and penis of the KS affected twin only, as well as in KS boys evaluated in childhood, were small at birth and throughout adolescence (5). Gonadotropins have generally been normal (low) in childhood and tend to rise by age 12-14 years in KS boys, similar to normal boys. However, normal gonadotropins in childhood do not necessarily indicate normal testicular function. Gonadotropins in girls with Turner syndrome who have early and severe ovarian failure are also normal in childhood and rise at age 10-12 years (6).

Other signs of testicular failure in adolescence include gynecomastia, eunuchoidal body proportion with relatively longer legs and increased arm span, decreased facial, pubic, and body hair, decreased muscle mass, and feminine distribution of body fat. Testosterone levels measured prenatally and in infancy in a small number of KS males were decreased or normal (7) and remained low-normal in childhood and adulthood (5). Testicular biopsy specimens of KS infants demonstrate decreased germ cells. After puberty, hyalinization and fibrosis of the seminiferous tubules occurs with small testes and

azoospermia. Thus, the extra X chromosome affects both testicular germ cells and interstitial cells.

II. Physical phenotype

KS boys tend to be tall (average adult height of 186 cm) (8), starting in infancy and childhood, with disproportionately long legs and arms and relatively small head circumference SDS compared to height SDS (4, 5, 9, 10). Their tall stature, mainly due to increased leg length, occurs in childhood and is likely related to 47,XXY males having a third copy of the X chromosome height determining gene, SHOX, as well as delayed epiphyseal fusion on the basis of decreased sex hormone levels. The SHOX (short stature homeobox) gene was first identified as a major human growth determinant from molecular analysis of sex chromosome abnormalities associated with short stature (11). Other sex chromosome trisomies, 47,XYY males and 47,XXX females, also tend be tall and have three copies of the SHOX gene (12). Skeletal anomalies associated with KS include osteoporosis, kyphosis, scoliosis, and pectus excavatum. KS males also have an increased risk for central obesity, starting in childhood (8).

III. Neurocognitive and behavioral phenotype

KS males also have a neurocognitive and behavioral phenotype that is expressed early in childhood and persists into adulthood. The main features include 1. language-based learning disabilities, 2. impaired working memory/executive function, and attention, 3. impaired motor abilities and 4. a characteristic personality style. Important information about the natural history of cognitive development in this population has been derived from several prospective, population-based longitudinal studies (8, 13-15). In general, global intelligence is less than siblings or controls (14), but Verbal IQ tends to be depressed relative to Performance IQ, both in children and adults (4, 14-18). In contrast, visual-spatial abilities are generally within the average range.

1. Language-based learning disabilities: KS children and adults have impairment of selective aspects of receptive and expressive language processing. Delayed language and speech development is often detected by age 2-3 (8, 19, 20). These delays are predictive of later school problems with reading, spelling, writing, and problem solving that necessitate special education in childhood and adolescence (16, 21). Detailed studies of these language-based learning problems suggest underlying deficiencies in perception and short-term memory for sequential auditory information, limitations of phonemic processing, and poor understanding of grammatical and morphological aspects of language. Associated expressive language problems include poor word-retrieval, depressed verbal fluency, and limitations in the capacity to formulate narrative constructions (14, 15, 18). These difficulties in articulating and structuring language output as well as impaired verbal processing speed, and executive abilities persist into adulthood (17, 22-24).

2. Working memory/executive and attention dysfunction: Neurodevelopmental anomalies of attentional systems are common. Attention deficits without hyperactivity (23,24) occur in KS males with executive dysfunction and problems with working memory. Androgen replacement in KS males has been associated with improved fluency (25). Also, we previously demonstrated that androgen replacement in girls with Turner syndrome (10-14 years) who have androgen deficiency on the basis of ovarian failure was associated with improved working memory.

3. Motor abilities: KS children and adults also have diminished neuromuscular integration with impaired gross motor and fine motor skills and coordination, speed, dexterity and strength (14, 26). Young KS infants frequently had decreased motor tone and atypical movement patterns (27) and in childhood, balance, jumping and hopping are impaired (27). In addition, finger joint hypermobility and poor grasp specifically hinder writing skills (26). The motor impairments lead to impaired eye-hand coordination, clumsiness and poor athletic skills, which in turn, have a negative impact on socialization and self-image.

Evidence of impaired simple motor function in girls with Turner syndrome (TS) and improvement with oxandrolone treatment for two years (Figure 1): We have included preliminary results from oxandrolone versus placebo treatment (0.06/mg/kg/day) of another androgen-deficient population: TS girls with ovarian failure, treated for 24 months [115]. Simple repetitive movement of the hand was assessed with subtests of the Physical and Neurological Evaluation for Soft Signs (PANESS). The time required to tap the thumb and forefinger 20 times and to tap the thumb to each of the 4 fingers sequentially 20 times was measured separately in the dominant (right only) and nondominant (left only) hands. Results were converted to SD scores using data from age- and handedness-matched control females. The change in SD scores at year 1 and year 2 are shown (Figure 1). The androgen-treated TS group had significantly improved simple repetitive movement of the hand ($P < 0.05$) after 24, but not 12 months, compared to the placebo group. The mechanism of androgen effects may be through organization of neuronal processes and /or their growth and efficiency, perhaps in the pyramidal and nonpyramidal areas. We propose to use the same treatment duration (24 months) in the KS population because statistically significant changes in this simple motor task were observed after 24 but not 12 months of treatment.

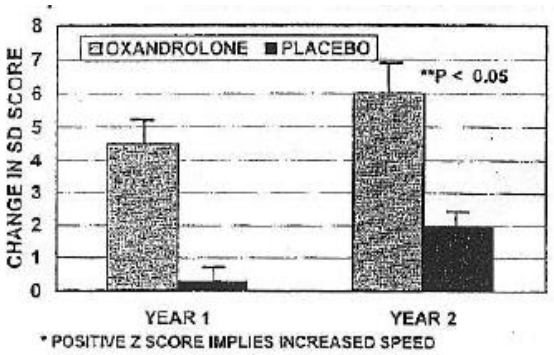

Figure 1: Change from baseline in simple repetitive movement SD Score* in girls with Turner syndrome treated with oxandrolone or placebo (X ± SEM) for 2 years *sum of PANESS 1-finger, 4-finger, dominant and nondominant hands

4. Personality development: There is also a psychosocial aspect of the KS phenotype affecting temperament and self-image. By age three, KS boys are relatively quiet, passive, and socially withdrawn. They have increased worries and fears as well as difficulties with peer and sibling relationships (4). Their quiet, shy, and unassertive personalities place them at an increased risk for being bullied. Many boys have diminished self-esteem, as early as age 7, as well as diffuse anxiety and immaturity. Adolescents remain shy and immature with increased difficulty making social contact with others and decreased sexual libido (28, 29). During adolescence, KS males were more prone to impulsivity, outbursts, and conflict than controls (30), suggesting that this is a particularly difficult time for them. When adults have been followed long term, the cognitive and behavioral findings remain, but there is no increase in criminality, major psychopathology, or homosexuality, compared to hypogonadal 46,XY males (8, 22, 31).

Certain psychiatric disorders including anxiety, depression, and psychosis are more common in KS (31) as well as a decreased likelihood for marriage. One study comparing KS adult males with 46,XY hypogonadal males reported increased immaturity, fatigue, social isolation, and delayed sexual milestones in the KS population (22). Both populations were androgen-deficient, but the deficiency may be more severe or longstanding in the KS population.

Poor motor coordination and athletic abilities impair normal development of positive self-esteem and increase anxiety and social isolation. The potential relationship of these social findings to the cognitive abnormalities is of great interest. Also, the contribution of androgen deficiency to this physical and social profile is unknown. Early testosterone replacement may increase muscle strength and coordination and may aid peer identification, self-esteem, and social function.

IV. Determinants of brain development in KS males

There appear to be early genetic effects of the additional X chromosome on brain growth and development, resulting in relative microcephaly at birth in KS males that persists and correlates with IQ. Support for relative microcephaly being related to genetic rather than hormonal mechanisms comes from the genetic model of 47,XXX females (4, 32) who are not androgen-deficient, but also tend to have relatively small heads. In addition, both KS males and 47,XXX females have increased speech and language problems, decreased coordination, and increased academic difficulties (12).

Neuroimaging studies of KS males have shown reduced left temporal gray matter, consistent with the verbal and language deficits, and reduced amygdala volumes which may be related to psychopathology or behavioral dysfunction of KS males (25, 33). The amygdala influences neuroendocrine, cognitive, and emotional aspects of information processing and is richly endowed with androgen and estrogen receptors. The volumes of the lateral ventricle and the amygdala increase with age in boys, particularly at puberty (34). This particular finding may provide a neuroanatomic basis for the KS shy temperament and passivity, while their poor phonemic processing and auditory memory may be related to alterations in left temporal lobe morphology (25).

Language ability is asymmetrically organized in the brain, with nearly all right handers and 50% of left handers demonstrating left hemisphere dominance. Corresponding volumetric asymmetries of the left perisylvian cortex include a larger left planum temporale which may relate to increased neural representation devoted to the processing of sound-based representations of speech (35). Geschwind and Galaburda proposed that cerebral dominance for language is related to prenatal testosterone levels (36, 37). Neuropsychological findings in KS males (38-40) suggest anomalous development of cerebral hemispheric asymmetry with greater right hemisphere involvement, perhaps related to slower fetal brain growth rates and the failure of the left hemisphere to gain dominance in language processing (40). Phonemic event related potentials (ERP), which reflect increased processing time for linguistic information correlate with verbal IQ in KS (38). These relationships lead to the speculation that the slower processing of the phonemic ERP reflects anomalous neural processing of speech related to anomalous cortical localization.

The etiology may be androgen deficiency in utero and early in life, excess X-chromosome gene dosage effects, or both.

V. Testosterone effects on the brain, cognition, and behavior.

Testosterone affects normal brain development in males and, in several models of androgen deficiency, androgen replacement improves specific aspects of cognition. Clearly, testosterone has organizational effects on the brain, both in utero and later in childhood and adulthood. Testosterone may also influence brain development through peripheral aromatization to estradiol. Whether reading and language deficits in KS are related to early androgen deficiency as opposed to genetic effects of the extra X chromosome is uncertain. That many KS infants are born with small phalluses and undescended testes would indicate early, in utero, testosterone deficiency. Another important testosterone effect on brain development may occur in infancy when there is normally a surge in testosterone, peaking during the second to fourth month of life in normal infant males. KS infants would presumably be lacking this testosterone surge.

Both animal and human studies have shown clear-cut structural effects of androgen on subcortical nuclear regions such as the hypothalamic/preoptic area as well as forebrain regions that are related to behavior (41-43). Androgen alterations during the perinatal period and puberty influence cognitive function and behavior in animal and human models. Gonadal steroids during human fetal development also affect cerebral dominance development and may lead to certain gender differences in cognition. Numerous examples support an association between androgen and spatial ability and to a lesser extent, working memory, in humans. Males generally outperform females in visual-spatial tasks involving mental rotation, spatial perception, spatial visualization, and problem solving, which all rely to some extent on working memory (44). Neuropsychological impairment occurs in subjects with androgen deficiency. Men with untreated, congenital, hypogonadotropic hypogonadism (IHH) have diminished production of testosterone as well as impaired spatial ability, verbal memory and attention relative to normal controls (45). Yet, clear differences remain between 47,XXY KS males and 46,XY males with IHH, with the language- based learning disabilities being more characteristic of KS.

Androgen replacement in KS males has been associated with improved fluency (39) and relatively increased left temporal cortical volumes (25). Working memory has been also been shown to respond to testosterone in elderly men who receive replacement therapy (46).The cerebral cortex may remain plastic until adulthood, permitting changes to be induced by testosterone. Against early testosterone deficiency being the sole etiology for the KS cognitive phenotype is the model of androgen-deficient males with hypogonadism but normal chromosomes, who do not generally suffer from dyslexia or impaired language abilities.

In contrast, the association between the self-image and social phenotypes in KS and androgen deficiency is stronger. Androgen replacement may help in the areas of distractibility, mood, and psychosocial function (22, 47, 48). Verbal IQs in KS adolescents were positively related to earlier pubertal onset and higher testosterone levels (49). Most KS adults treated with testosterone benefited, reporting improved endurance and strength, concentration, learning ability, and mood (22). In addition, testosterone replacement in normal males with delayed puberty has been associated with improved self-perceived competence (47). What is not yet clear is what the optimal age for initiating testosterone treatment in KS males would be.

VI. Genetic mechanisms

The extra X chromosome in KS is usually acquired though non-disjunction during maternal or paternal gametogenesis, occurring in the egg (40-50%) or the sperm (50-

60%), or through an error in mitotic division in the zygote (50-52). Maternal inheritance of the extra X results from non-disjunction in the first (70%) or second (20%) meiosis or as a post-zygotic mitotic error (10%), with increased maternal age and reduced recombination also occurring (51).

VII. Early androgen replacement in KS

In summary, the hormonal and genetic contributions to the KS phenotype remain to be defined more clearly. Early diagnosis, together with early parental education and counseling, and earlier testosterone replacement may contribute to positive adjustment and reduce the negative social, emotional, and education impact of KS. Further studies linking the hormonal and genetic mechanisms will increase our understanding of the pathogenesis of KS and will permit more specific genetic counseling and interventions.

The primary goal of this study is to test the effect of replacing the low levels of androgen in KS boys in an attempt to mimic the testosterone production that occurs normally in boys. Clinical support for androgen deficiency occurring earlier in childhood in KS boys than previously thought comes from the poor growth of the penis throughout childhood in these boys. The "standard of medical care" for initiating testosterone replacement therapy in KS males has typically been at age 11 or 12 years or older (59). Testosterone treatments in KS teenagers or adults results in appropriate virilization with increased muscle mass, more masculine body contour, increased body hair, penile growth, and amelioration of eunuchoidal tendencies.

Typically, adult replacement doses of 200 mg of testosterone esters such as testosterone enanthate or cypionate are given every two weeks and newer formulations utilizing the intradermal or oral route are also available in relatively high adult-level, replacement dosages. There are relatively few options for lower dose androgen replacement. One attractive consideration is the oral androgen oxandrolone which has been used safely in boys with delayed puberty and in girls with Turner syndrome for over twenty years and is FDA approved for children and adults for the indication of wasting (60-62). Oxandrolone safely increased muscle mass in prepubertal boys with constitutional delay of growth (63). We propose a two-year clinical trial using two doses of oxandrolone versus placebo in young KS boys, ages 4-12 years (see below).

RESEARCH DESIGN

Study Design: This study consists of a clinical trial composed of an eligible subset of KS boys ages 4-12 years. The randomized design excludes bias in the assignment of patients to the treatment group and ensures optimal matching of the treatment group, which includes: 1. Oxandrolone (0.06 mg/kg PO, daily), or 2. oral placebo. The PI, patients, and research assistant will be blinded as to whether the oral medication is oxandrolone or placebo. The randomization is performed by the research pharmacy.

Study Population: For the clinical trial, KS boys ages 4-12 years will be randomly assigned to one of two treatment groups (androgen versus no androgen). We will recruit 180 males (60 per year for 3 years) assuming an approximate 30% drop out rate, to accrue a total of 120 patients for the clinical trial substudy.

Patient Recruitment: The PI has the unique resource of a clinic at Thomas Jefferson University, where 60 KS boys are followed. All are untreated with any androgen. We will also recruit additional patients by notifying patient groups and physicians about the study.

PROCEDURES OF THE STUDY

I. Inclusion Criteria:
1. Karyotype diagnosis of Klinefelter's syndrome or Klinefelter's variants, including 48,XXYY and 48,XXXY
2. Chronological ages 4 to 12 years
3. No previous treatment with androgen or estrogen in past one year

II. Exclusion Criteria:
1. Major liver, kidney or other systemic disease
2. Dual diagnoses including pervasive developmental delay or autism.
3. Variant karyotypes including 46,XX males and 47,XYY males
4. A high level of mosaicism (more than 50% 46,XY cells)

III. Study Outcome:

Physical features

We will document visible phenotypic features using a digital photography system. Specific physical features examined and tests used are as follows:

1.Anthropometric measurements

1. Measurements. The clinical assessment will include measurement of height, lower segment, and arm span. Subjects' heights will be measured with a stadiometer. Measured or reported parental heights will also be recorded. Mid-parental height adjusted for the child's sex (target height) will be calculated using the formula 0.5 x [father's height (cm) + mother's height (cm) + 13] (64). Target height z-scores are calculated for subjects from sex-adjusted mid-parental height obtained from the National Center for Health Statistics growth curve data (65). Arm span will be measured as the distance from right to left 3rd finger tips with patients facing the wall, with outstretched arms held parallel to the ground. Lower segment will be measured from the top of the symphysis pubis to the floor. Upper arm and lower leg circumferences will be measured. Results were converted to z-scores where possible using age- and gender-specific norms (65-67).

2. Genitalia. We will measure penile length. Standards are available for penile growth in childhood and percentiles will be recorded (68). In addition, testicular size will be assessed with the Prader orchidometer and the measured volume will be recorded.

3. Optional MRI SCAN: Patients with KS will be asked if they would have an MRI scan done at baseline and at or between the 18 and 24 month visits, when they are participating in the clinical trial.

The pathophysiology of KS includes changes in brain morphology and hemodynamic events. The standard clinical MRI techniques do not provide functional information regarding perfusion, oxygenation and functional connectivity in the brain. It is observed that physiological, cognitive and functional changes occur in addition to anatomical changes detectable using conventional protocols. This is usually associated with abnormal vascularity and localized functional abnormalities. In addition, white matter integrity and brain circuitry may also be affected. Therefore, the ability to assess oxygenation, perfusion and functional connectivity and their changes under available

therapies in the brain of KS boys will greatly facilitate characterizing their stage of development, assessing their cognition and monitoring treatment.

The goal of this procedure is to use a suite of complementary noninvasive, quantitative and functional MRI techniques to investigate cerebral blood supply, brain activity patterns and functional brain connections in patients with KS treated with Androgen or placebo, as part of the clinical trial. Patients will be excluded if they have claustrophobia, pacemakers, metallic clips in the body, or orthodontic appliances or braces in the mouth. The procedure does not hurt or include any intravenous access or injections. No sedation is involved.

Laboratory studies

1. Testicular function. Serum testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH), and salivary testosterone levels will be measured. Testicular failure is defined by castrate levels of FSH and LH in subjects at least ten years of age. Typically, the gonadotropins are suppressed in agonadal KS boys during childhood and do not rise until after age 10. Therefore, serum gonadotropin levels may not be a reliable index of testicular failure before age 10 years. Testicular function in boys <10 yrs. old will be considered indeterminate if the gonadotropins are not elevated and will be reassessed in those subjects who reach age 10 by the end of the study. The presence of early testicular failure will be determined by measuring penile length and testicular size.

2. Thyroid function. Free thyroxine (free T4) and thyroid stimulating hormone (TSH) will be measured because of an increased risk for thyroid disorders in KS. Standard criteria of the Jefferson clinical laboratory will be used to determine whether tests are abnormal.

Radiographic studies

1. Skeletal x-rays of the hands will be obtained for all subjects. Bone age will be determined according to the method of Greulich and Pyle (69).

Cognitive Outcome summary scores (To be age-appropriate):

1. General Cognition summary score (KS males, ages 2-60)
    a. The Differential Ability Scales-(DAS)
2. Language summary score (age-appropriate):
    Phonological
    a. Comprehensive test of Phonological Processing (CTOPP).
    . Verbal Memory
    a. California Verbal Learning Test-Children's Version (CVLT)
    b. Children's Memory Scale (CMS)-story memory.
    Expressive
    a. Expressive one word vocabulary test
    b. Rapid naming (NEPSY)
    c. Test of language Competence (TLC) – Oral Expression
    Receptive
    a. Peabody Picture Vocabulary Test (PPVT)
    b. Token Test
3. Working memory/Executive function/Attention summary score
    a Digit span backwards (DAS)

b. Connors' Continuous Performance Test II (CPT II) and Kiddie Version (K-CPT)
c. Connors' Rating Scales-Revised
d. Verbal Fluencies-NEPSY(semantic)
e. Design fluency-NEPSY

4. Motor function summary score

Strength
a. Bruininks-Oseretsky Test of Motor Proficiency

Fine motor
a. Bruininks-Oseretsky Test of Motor Proficiency (BOTMP)-Fine Motor Composite

Gross Motor
a. Bruininks-Oseretsky Test of Motor Proficiency (BOTMP)-Gross motor composite
b. NEPSY-praxis

5. Self-image summary score-Males less than 21 years of age

Social Function (Competence)
a. Behavior Assessment System for Children (BASC)
b. Child Behavior Checklist (CBCL)

Affect and Behavior
a. Revised Child's Manifest Anxiety Scale (RCMAS)

IV. Clinical trial substudy: Boys with Klinefelter's syndrome will enter the study at ages 4-12 years. At baseline, they will have a history and physical examination, cognitive and behavioral assessment, and further genetic evaluation. All potential subjects will have a full screening to determine eligibility before randomization. They will be randomized (double-blind) to one of two treatment groups: oxandrolone 0.06 mg/kg/day, or placebo control for two years. All subjects will be evaluated for safety at baseline, 3, 6, 12, 18 and 24 months, and after one and two years for end-point efficacy (cognitive and behavioral assessment).

The KS boys will be seen at 3-6-month intervals for the two-year duration of the study. On each visit, boys will receive a careful history and physical examination to include assessment of Tanner staging (73) for pubic hair development (Tanner 1 = prepubertal-Tanner 5 = fully mature) and measurement of penile length and testicular volume. At baseline, three, and every six months thereafter, we will measure height, weight, bone age (X-ray); fasting blood work to include complete blood count, serum glucose, liver function tests (SGPT), lipid panel, thyroid studies, testosterone, and free testosterone (the active form of testosterone) levels. LH, FSH and estradiol will be measured yearly. At baseline and after one year, each boy will undergo a battery of cognitive tests that will take approximately 5 to 7 hours (see below).

The form of androgen will be the oral, nonaromatizable androgen oxandrolone. This androgen is FDA-approved for the indication of wasting and has been used safely for the past 30 years to stimulate growth in girls with Turner syndrome without virilization or major side effects. The dose, 0.06 mg/kg/day, was chosen because of the extensive previous experience with the dose of 0.06 mg/kg/day used in girls with Turner syndrome. Children will be monitored closely for signs of virilization including acne, voice changes,

development of pubic hair, and excessive bone age advancement. Since oxandrolone is nonaromatizable and cannot be converted to estradiol, we do not expect any feminizing side effects such as breast development to occur.

Safety results necessitating an approximate 10-50% reduction in medication dose or stopping the medication:

1. Bone age advancement exceeding 12 months per 6-month interval, with bone age exceeding chronologic age.
2. Signs of early pubertal development for age, including pubic hair development in boys less than 8 years of age.
3. Systolic blood pressure exceeding 95th %ile for age.
4. Diastolic blood pressure exceeding 95th %ile for age.
5. LDL cholesterol greater than159 mg/dl.
6. HDL-C less than 20 mg/dl.
7. SGPT exceeding twice the upper limit of normal.
8. Dose can be lowered at the discretion of the investigator for other reasons such as rapid growth.

The oxandrolone dosage cannot be reduced by precisely 50% because of the nature of the oxandrolone tablets that come in a single strength, 2.5 mg. The 2.5 mg pill is scored and could be accurately split to the 1.25 mg dose. The 1.25 mg dose cannot be accurately split further. Thus, according the criteria listed above, we could reduce the 3.75 mg dose to 2.5 mg and the 2.5 mg dose to 1.25 mg. Dose reduction will therefore range from approximately 10-50%, depending on the weight of the child. If the dose needs to be reduced to less than 1.25 mg/day, we will change from daily to every other day dosing.

If at any visit the child's weight is greater than the 95th percentile, the 95th percentile weight in kg will be used to calculate the dose. If this dose reduction is implemented, then additional reductions would not be implemented at that visit for the other dose reduction criteria. At a single visit, if the dose of oxandrolone is reduced for one criterion, then the dosage is not reduced again for another study criterion.

Safety results necessitating stopping the study:

If at any point in the study, two or more patients develop severe liver function changes or hypertension, the study will be stopped. These changes have not occurred with oxandrolone used in a trial with 73 girls with Turner syndrome followed for up to four years and are therefore not expected.

Criteria for Subject Withdrawal from the study and adverse event reporting:

Subjects can withdraw from the study at any time for any reason. The PI may terminate a subject's participation due to severe adverse events, noncompliance with the study, or concerns about safety. Safety data will be reviewed on an ongoing (blinded) basis by the PI.

Study compliance: We will assess patient compliance by having the families fill out study cards when they administer all medications and return the cards as well as all used and

unused study materials at the time of their visit. All pills dispensed and returned will be counted and recorded. We are currently using this procedure in other ongoing studies and have found that it is effective.

Post-Study Visit: Per the recommendation of the Data Safety and Monitoring Board (DSMB) all study participants will be invited back for a post-study visit. This visit will occur approximately 6-28 months post-study for patients who have enrolled in the study. The post-study visit will include a history, physical examination, fasting blood work, bone age x-ray, photographs, and cognitive testing. Patients will be reimbursed $50.00 for completion of the visit. Patients who are unable to travel to a post-study visit or who live farther than driving distance will be asked to have a blood sample drawn locally and sent to our office in Philadelphia.

ANALYSIS

The analysis will be performed in consultation with the statistician, Dr. Harvey Kushner who has been collaborating with Dr. Ross for the past 15 years. The analyses described below will include 2 groups of KS boys. All results will be based on gender and age-specific standardized scores. Analysis for safety will be performed at 3, 6, 12, 18, and 24 months and an efficacy analysis for summary scores encompassing all cognitive and behavioral variables will be performed at 12 and 24 months.

I. Safety: An analysis of safety parameters will be performed for the 3, 6, 12, 18 and 24 month values based on a two-way ANCOVA, adjusting for baseline values, comparing the active treatment group versus the placebo. The safety parameters are: systolic and diastolic blood pressure, HDL, LDL cholesterol, liver function (ALT), bone age advancement, or signs of puberty (pubic hair).

II. Primary efficacy (Hypotheses 1 a,b,c): The analysis comparing androgen-treated to placebo-treated KS subjects for the one and two-year measurements will be based on the analysis of covariance (ANCOVA). We will perform a two-way ANCOVA for the five primary outcome measures at 12 and 24 months, comparing the treatment group versus the placebo, adjusting for baseline values and socioeconomic status. There will be a total of 5 summary scores. Each score will be the sum of component z-scores based on gender and age-specific normative values. The summary scores include 1. General cognition, 2. Language, 3. Working memory/executive function and attention, 4. Motor function, and 5. Self-image and behavior. The alpha level for statistical significance will be set at 0.01 for each of the five primary outcome results.

Data Management and processing: Dr. Harvey Kushner, the biostatistician, will perform all statistical analyses. All data will be entered using a custom program written for the project. An on site data manager will oversee the data collection and entry, and quality control. The database will be visual dBase for data entry and will be converted to SAS (version 8.2) for statistical analyses. Dr. Kushner will produce the data source tables, summary data tables as well as perform the designated analyses. All entered data will be manually compared to the source documents for accuracy. Additional checks for consistency within and between records will be built into source documents for accuracy. Baseline and follow-up data will be collected on case report forms.

Power Analysis

This RCT is based on a randomized block design, comparing the treatment group with the placebo group composed of males who all have Klinefelter's syndrome and are between the ages of 4 and 12 years of age. Based on previously published data for word fluency performance in KS males, receiving or not receiving testosterone (25), we performed a power analysis and determined that we will have > 90% power, at alpha = 0.05, two-tailed, to detect significant androgen effects on working memory/executive function for each active treatment group versus the placebo group, with n=20 in each group.

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