

RESEARCH REVIEW

Morbidity in Klinefelter syndrome and the effect of testosterone treatment

Simon Chang^{1,2,3} | Anne Skakkebaek^{4,5} | Shanlee M Davis^{6,7} |
Claus H Gravholt^{1,5}

¹Department of Endocrinology and Internal Medicine, Aarhus university Hospital, Aarhus, Denmark

²Department of Internal Medicine, Lillebaelt Hospital, Kolding, Denmark

³Unit for Thrombosis Research, Hospital of South West Jutland, Esbjerg, Denmark

⁴Department of Clinical Genetics, Aarhus University Hospital, Aarhus, Denmark

⁵Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark

⁶Department of Pediatrics, University of Colorado School of Medicine, Aurora, Colorado

⁷eXtraordinary Kids Clinic and Research Program, Children's Hospital Colorado, Aurora, Colorado

Correspondence

Simon Chang, Department of Internal Medicine, Lillebaelt Hospital, Sygehusvej 24, DK-6000 Kolding, Denmark.
Email: simon.chang@rsyd.dk

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Abstract

Klinefelter syndrome (KS; 47,XXY) is the most common sex chromosome abnormality in males (150 per 100,000 males). The condition leads to hypergonadotropic hypogonadism and ever since the condition was described approximately 80 years ago, testosterone treatment has been the cornerstone in care for individuals with KS. However, KS is associated with an array of health-related and socioeconomic challenges and it is becoming progressively clear that proper care for boys and men with KS reaches far beyond simply supplementing with testosterone. There are no widely implemented guidelines for KS care, and studies investigating crucial aspects of testosterone treatment in individuals with KS, including both beneficial and potentially adverse effects, have only begun to emerge during the last decades. For this descriptive review, we present an overview of literature describing health-related outcomes of testosterone treatment in KS and outline the clinical applications of testosterone treatment in KS. Collectively, beneficial effects of testosterone treatment on overall health in KS are described with few apparent adverse effects. However, larger randomized studies in adult and pediatric patients are warranted to elucidate key aspects of treatment. We stress the implementation of centralized multidisciplinary clinics and the need for a dedicated international guideline to ensure optimal care of boys and men with KS.

KEYWORDS

hypogonadism, Klinefelter syndrome, review article, testosterone treatment

1 | INTRODUCTION

Taking care of boys and men with Klinefelter syndrome (KS; 47,XXY) presents itself with a number of challenges. Despite being present in approximately 1:600 of male births (150 per 100,000 live born boys) (Berglund et al., 2019; Coffee et al., 2009; Herlihy, Halliday, Cock, & McLachlan, 2011), the diagnosis is only made in around 25% of the expected number of cases and commonly not until well into adulthood, with a median age at diagnosis of 27 years (Abramsky & Chapple, 1997; Berglund et al., 2019; Herlihy, Halliday, et al., 2011).

Secondly, growing up and living with KS is associated with an increased risk of a wide array of comorbidities, necessitating prophylactic and specific treatments with inclusion of an equally diverse group of health care professionals from many different specialties (Gravholt et al., 2018). Finally, it is evident that being born with KS is associated with socioeconomic and neurocognitive challenges resulting in a need for specialized guidance and support (Gravholt et al., 2018; Skakkebaek, Wallentin, & Gravholt, 2015; Stochholm, Juul, & Gravholt, 2012). Although the additional X chromosome has global implications, one of the consequences of KS is testicular

dysfunction resulting in hypergonadotropic hypogonadism. Therefore supplementation with exogenous testosterone is at the core of KS care. This review seeks to outline the current knowledge about how supplementation with testosterone is being carried out in boys and men with KS and to present available evidence related to the health effects of testosterone supplementation in KS. The available literature has been scrutinized and selected publications were included at the authors' discretion for this descriptive review. For data regarding neurocognitive function in individuals with KS, refer to the dedicated review presented in this same journal issue covering this topic.

2 | SHOULD INDIVIDUALS WITH KS BE TREATED WITH TESTOSTERONE?

It might seem obvious that a condition resulting in male hypogonadism should be treated with testosterone supplementation. However, from clinical experience, not all individuals with KS are offered such treatment (Chang, Bilotto, et al., 2019). Patients referred to our clinic often describe how physicians aware of the diagnosis of KS have been unknowing about the need for testosterone supplementation (Chang, Christiansen, et al., 2019). In the original publication describing KS, it is stated that "testosterone therapy is probably indicated," but the authors also reported little effect of high-dose testosterone treatment on the visual presentation of the index cases, particularly regarding the size of testes and gynecomastia (Klinefelter, Reifenstein, & Albright, 1942). Today we know that male hypogonadism is associated with numerous unfavorable physiological changes and development of several more or less visible conditions, such as anemia, osteoporosis, body composition changes, and mental health disorders (Bhasin et al., 2018). As such, major guidelines for testosterone treatment all agree that hypogonadism in men should be treated (Salter & Mulhall, 2019). However, these guidelines are not specific to the primary hypogonadism that is characteristic of KS, nor do they address hypogonadism and testosterone treatment during puberty. As testosterone concentrations in an adolescent or young man with KS may still be in the low normal range although often with elevation of gonadotropin levels becoming evident, the criteria for initiating testosterone treatment given in most guidelines, for example, repeated low testosterone levels or symptoms of hypogonadism, represent a reactive approach that may lead to insufficient treatment in KS. However, whether long-term testosterone supplementation in KS is capable of preventing comorbidities, improving neurocognitive function and quality of life, and decreasing mortality in KS is still largely unanswered. At the present time, clinicians with expertise in managing KS generally agree that testosterone treatment is beneficial for most men and adolescents with KS who have elevated luteinizing hormone and low (or low-normal) serum testosterone concentrations, although there are certainly those that think that testosterone levels should fall below the normative range before commencing therapy. There continues to be disagreement as to if testosterone treatment in the absence of hypergonadotropism, including boys with KS prior to and in early puberty, is clinically indicated.

3 | HOW SHOULD WE TREAT INDIVIDUALS WITH KS WITH TESTOSTERONE?

In today's clinical practice, treatment of patients with disease should preferably follow international guidelines representing the best practice advice based on condensation of the available research by leading experts. This approach increases the likelihood for a given patient of receiving the best possible care. To our knowledge there are no widely accepted specific guidelines for overall care of KS or even for testosterone supplementation in KS. As an example, the current guideline for testosterone treatment from the Endocrine Society only mentions KS as being a cause of primary male hypogonadism, but gives no directions as how to treat this specific patient group, when to start treatment, which route to use when choosing testosterone supplementation, which dose to use, which target tissues to monitor, how often to monitor, or which treatment goals to use to declare treatment a success or not (Bhasin et al., 2018). Naturally, it can be argued that hypogonadism is hypogonadism and that males with KS can be treated by the same guidelines as everyone else. However, KS is a congenital condition and in our opinion the early onset and long-term hypogonadism in KS differs significantly in both severity and derivative health effects, compared with the relative lack of testosterone seen with increasing age. Along with the many other specific aspects of care in KS, we find that there is a genuine need for a dedicated guideline, preferably based on an international collaboration. Such a guideline would underline the need for implementing highly specialized centers facilitating interdisciplinary team work, including expertise from fields such as clinical genetics, pediatrics, adult medicine, surgery, cardiology, psychiatry, as well as educational and psychosocial specialists. In regard to testosterone supplementation, a future guideline should also give specific aims for treatment in KS, preferably by evaluation of measurable predefined endpoints or intermediate endpoints, such as the level of luteinizing hormone (LH), testosterone, hemoglobin, bone mineral density, quality of life measures, libido, cognitive functioning, learning ability, and so on. This would not only allow for qualitative assessment of treatment, but would also provide a helpful tool for patients regarding what to expect from initiation of treatment. In absence of a specific guideline for clinical management in KS, the way testosterone treatment is implemented is highly variable and dependent on clinician experience, patient preferences, and availability of formulation and possibly also insurer's perception of the role of treatment, rather than on data-driven safety and efficacy. As a general rule, testosterone treatment should aim to restore but not exceed physiologic systemic testosterone levels. Patients should be educated about the positive effects and potential side effects of treatment and formulation options to participate in treatment decisions.

4 | TESTOSTERONE TREATMENT AS A MODIFIER OF DISEASE RISK IN KS

Despite the apparent need to treat hypogonadism with testosterone in individuals with KS, there are relatively few studies directly

addressing the health effects of treatment, and most of these studies are cross-sectional or uncontrolled longitudinal studies. In the following, we present a condensation of the available literature reporting health outcomes of testosterone treatment in KS in adults, and, when available in boys and adolescents.

4.1 | Body composition

KS is associated with increased body fat and higher rates of metabolic disorders such as Type 2 diabetes and the metabolic syndrome (Gravholt et al., 2018). Progressive hypogonadism seems to drive a process leading to deposition of adipose tissue and insulin resistance that, as part of a vicious cycle, further aggravates hypogonadism in KS (Gravholt et al., 2018). It is, however, uncertain to what extent the metabolic changes seen in KS are directly associated with the hypogonadal state and to which degree other factors, including direct genetic effects due to chromosomal imbalance, are contributing.

Although pediatric studies are small in number, abnormal body composition with higher adiposity and lower lean mass seems to begin early in life in males with KS. A small study of body composition using air displacement plethysmography in 10 infants with KS found that percent body fat was 1 SD above the mean of typical males at 5 months of age (Davis, Reynolds, Dabelea, Zeitler, & Tartaglia, 2019). In a sample of 93 prepubertal boys 4–12 years, body fat by skin folds was approximately 1 SD above the mean, confirming that adiposity was high despite a normal body mass index (BMI) (Davis et al., 2016). Cross-sectional studies consistently find that testosterone treatment in men with KS is associated with a reduction in fat mass and total body fat percentage (Bojesen, Kristensen, et al., 2006; Chang et al., 2015; Chang, Biloft, et al., 2019; Granato et al., 2019; Jorgensen et al., 2015; Pasquali et al., 2013). As an example, in our most recent cross-sectional study, total body fat in testosterone-treated men with KS was 20% lower in treated compared with an age-matched group of untreated men with KS (Chang, Biloft, et al., 2019). However, body fat in treated men with KS was still higher than in control men, perhaps indicative of insufficient testosterone replacement therapy or that testosterone replacement therapy was not given for a prolonged period of time, or finally that a longstanding period of hypogonadism during puberty and early adulthood leads to a sort of metabolic memory which then could be perceived as a kind of relative resistance to the effects of testosterone treatment. As an example, the effects of testosterone treatment on metabolism seem to be more pronounced in men with idiopathic hypogonadotropic hypogonadism compared with men with KS (Jiang-Feng et al., 2012), although direct head-to-head studies comparing similar dose and duration of testosterone replacement therapy in males with KS and other groups of hypogonadal men have not been performed. Also, typically in cross-sectional and uncontrolled studies, no change is seen in BMI after testosterone treatment in KS, possibly due to counterbalancing effects of fat loss and muscle build up (Bojesen, Kristensen, et al., 2006; Chang et al., 2015; Granato et al., 2019; Selice et al., 2013). The anabolic effect of testosterone has even been associated with

increasing BMI after testosterone supplementation in two longitudinal studies (Jiang-Feng et al., 2012; Yesilova et al., 2004).

Recently, the effect of testosterone (or other androgen) treatment on body composition in individuals with KS has been evaluated in three placebo-controlled randomized trials in different age groups (Host et al., 2019). In the adult study, 13 men with KS (age: 34.8 years; BMI: 26.7 kg/m²) were included in a double-blind, placebo-controlled, BMI-matched cross-over study, applying oral testosterone 160 mg per day or placebo treatment for 6 months. Distribution of fat was evaluated both by dual X-ray densitometry and computed tomography scans. Testosterone treatment versus placebo was associated with significant reductions in total body fat and total abdominal fat, the latter driven mainly by loss of subcutaneous abdominal fat while no effect was observed on intra-abdominal fat (Host et al., 2019). Two randomized clinical trials of androgen treatment have been conducted in prepubertal boys with KS. The first was a 2-year, randomized, placebo-controlled trial of oral oxandrolone in 93 prepubertal boys 4–12 years of age (Ross et al., 2017). Oxandrolone was used here because it is a nonaromatizable androgen, which only minimally is converted to estrogen, therefore minimizing the effect of estrogen on bone age advancement. This study reported a significant decrease in adiposity in the treated boys as measured by skin folds but adiposity was still above average for age (Davis et al., 2017). The other trial was conducted in infants with KS. Normally, there is a temporary activation of the hypothalamic–pituitary–gonadal axis in infancy where males produce high concentrations of testosterone (the so-called minipuberty; Rey, 2014). A pilot, randomized study of 20 infants with KS found a significant difference in body composition between those treated with 3 months of testosterone injections (similar to control males) compared to those untreated (increase of 1 SD in adiposity) (Davis et al., 2019).

Together these studies support that total body fat is elevated in males with KS compared with age-matched controls with an accumulation of abdominal adiposity in particular. Testosterone treatment does seem to improve body composition based on these limited studies; however, given the anabolic effects of androgen, it is possible that the effects on body composition seen in these studies of boys and men with KS are not unique to individuals with KS. It is also unknown whether intervention with androgen yields lasting benefits on body composition or other health outcomes and longer term intervention studies are needed.

4.2 | Glucose metabolism

As already mentioned, metabolic syndrome and diabetes lead to increased morbidity for many men with KS, therefore glucose metabolism and insulin resistance are outcomes of interest with testosterone treatment. A recent study demonstrated that higher testosterone levels in 13 men with KS were associated with better insulin-mediated glucose disposal during a hyperinsulinemic euglycemic clamp (Yesilova et al., 2005). Furthermore, cross-sectional data have demonstrated reduced Homeostatic Model Assessment for Insulin

Resistance (HOMA-IR) among 97 testosterone-treated men compared with 35 untreated men with KS (Zitzmann et al., 2015). In the placebo-controlled randomized study by Høst et al., effects of testosterone treatment on glucose metabolism was addressed by applying a hyperinsulinemic euglycemic clamp with the glucose infusion rate during the last hour of the clamp acting as an index of insulin sensitivity, as well as the HOMA-IR. Under these circumstances, 6 months of testosterone treatment compared with placebo treatment was not associated with changes in insulin-mediated glucose disposal, insulin sensitivity or HOMA-IR between testosterone or placebo-treated men with KS (Host et al., 2019). Similarly, insulin sensitivity by HOMA based on fasting levels of insulin and glucose was not different in two cross-sectional studies comparing untreated and testosterone-treated men with KS (Bojesen, Kristensen, et al., 2006; Pasquali et al., 2013). Of note, testosterone levels in one study were increased more than 20% in the treated versus nontreated group (Pasquali et al., 2013), while testosterone levels between treated and untreated men with KS did not differ significantly in the other study (Bojesen, Kristensen, et al., 2006), speaking against clinically relevant direct association between testosterone levels and insulin sensitivity in KS. In contrast, a previous uncontrolled study of 56 men with KS demonstrated a significant reduction in HOMA-IR after 18 months of treatment with testosterone gel (Selice et al., 2013). Thus, given the conflicting results in these studies, it is not clear whether testosterone treatment in KS directly affects insulin sensitivity. It is likely the effect of testosterone treatment on insulin sensitivity would be secondary to loss of fat mass and increased muscle mass, rather than direct effects on glucose metabolism. Suffice to say, further studies are needed with longer treatment periods and larger number of males with KS males to fully uncover the effects of testosterone treatment on insulin sensitivity.

There are indications that insulin sensitivity in KS could be associated with specific genetic components (Gravholt et al., 2018), and it remains to be elucidated whether testosterone treatment in KS is effective in reducing the excess risk of Type 2 diabetes and the metabolic syndrome in KS (Bojesen, Juul, Birkebaek, & Gravholt, 2006; Bojesen, Kristensen, et al., 2006; Gravholt et al., 2018; Ishikawa, Yamaguchi, Kondo, Takenaka, & Fujisawa, 2008; O'Connor, Snyder, & Hayes, 2019). It is possible that chronic hypogonadism results in metabolic memory that cannot be easily erased or reversed with short-term correction of hypogonadism and could be an argument for appointing more resources to minimizing the diagnostic lag-time among most with KS and thus the time spend in a hypogonadal state. Additional investigation of the pathophysiology of the altered energy metabolism and long-term effect of androgen interventions is needed for boys and men with KS. Importantly, from Danish national registry data, men with KS redeeming testosterone prescriptions are also more likely to redeem prescriptions for antidiabetics compared with those men with KS with no testosterone prescriptions (Chang, Christiansen, et al., 2019). We believe this phenomenon, rather than being an effect of selection bias, is due to an overall higher standard of care in KS patients attending specialized clinics, thus leading to males being appropriately diagnosed with diabetes and receiving relevant treatment. As such, adherence to testosterone treatment could have a

secondary effect on risk of diabetes and diabetes control and by that potentially quality of life and mortality. More data are needed to support this hypothesis.

4.3 | Cardiovascular risk

Individuals with KS commonly present with a lipid profile associated with increased cardiovascular risk, namely high total cholesterol, elevated low-density lipoprotein (LDL) fraction, decreased high-density lipoprotein (HDL) fraction, and increased triglycerides (Garolla et al., 2018), but the impact of dyslipidemia on cardiovascular risk in KS is not known. Data from nine uncontrolled studies in adults (Aksglaede, Skakkebaek, Almstrup, & Juul, 2011; Bojesen, Kristensen, et al., 2006; Chang et al., 2015; Jiang-Feng et al., 2012; Jorgensen et al., 2015; Pasquali et al., 2013; Selice et al., 2013; Yesilova et al., 2004; Zitzmann et al., 2015) providing comparisons between lipid fractions among treated and untreated KS have previously been reviewed (Gravholt et al., 2018). Most find no effect of testosterone treatment on levels of total cholesterol or LDL cholesterol while two cross-sectional studies report higher triglycerides in treated KS (Chang et al., 2015; Jorgensen et al., 2015) and four cross-sectional studies reporting decreased HDL in treated KS (Aksglaede et al., 2011; Chang et al., 2015; Jorgensen et al., 2015; Zitzmann et al., 2015). A similar pattern was seen in our most recent cross-sectional study with lower HDL and a tendency toward increased triglycerides in 27 treated versus 18 untreated men with KS (Chang, Biloft, et al., 2019). Similarly, in the only randomized placebo-controlled study available, testosterone treatment was associated with an insignificant increase of triglycerides and decrease of HDL (Host et al., 2019). In 2011, Ross and colleagues published the results from 89 prepubertal boys 4–12 years of age with assessments of metabolic health biomarkers including fasting glucose, insulin, lipids, waist circumference, and blood pressure (Bardsley, Falkner, Kowal, & Ross, 2011). The majority of these young boys had low HDL cholesterol and 15% had elevated triglycerides. Eight percent met the full criteria for metabolic syndrome and another 36% met two of the criteria. When treated with oxandrolone, triglycerides decreased, however, HDL also decreased. If testosterone treatment causes lowered levels of HDL, then this could increase cardiovascular risk as by the Framingham Risk Score (D'Agostino Sr. et al., 2008). However, it is not clear whether the risk assessment given by the Framingham Risk Score applies to men with KS, and in particularly other beneficial effects of testosterone, including modulation of fat mass, could counterbalance any harmful effect of lowering HDL. To this end, a recent cross-sectional study found indications that testosterone treatment in KS could reduce epicardial fat, a novel marker of cardiovascular risk (Granato et al., 2019).

Changes in blood pressure from testosterone treatment would also potentially have an effect on cardiovascular risk. Most available studies from non-KS populations find an inverse association between testosterone and systolic blood pressure (Yang et al., 2019). In one study, 8 years of testosterone treatment was associated with a more

than 20 mmHg reduction in systolic and 15 mmHg reduction in diastolic blood pressure comparing 82 hypogonadal men treated with testosterone with 82 propensity-matched hypogonadal men who did not receive treatment (Traish, Haider, Haider, Doros, & Saad, 2017). However, data in KS are scarce. The pediatric oxandrolone trial did not report a change in systolic or diastolic blood pressure with treatment (Davis et al., 2017). In the recent placebo-controlled randomized study in 13 adults, testosterone was not associated with any changes in day, night, or 24-hr blood pressure (Host et al., 2019). Similarly, Foresta et al. (2012) saw no difference in blood pressure comparing 46 men with KS and normal testosterone levels with 46 men with KS and low testosterone levels. Contrastingly, we recently demonstrated higher systolic blood pressure among 27 men with KS treated with a median duration of testosterone treatment of 10 years compared with 18 untreated KS (Chang, Biloft, et al., 2019). However, typically blood pressure in KS is within the normal range (Gravholt et al., 2018), and whether any effect of testosterone treatment on blood pressure would be clinically relevant seems unlikely. However, in our recent cohort study assessing prescription medicine, men with KS on testosterone therapy were twice as likely to also receive prescriptions for antihypertensive medications compared with those men with KS not redeeming testosterone prescriptions (Chang, Christiansen, et al., 2019). We believe, as mentioned above, that this is a reflection of a higher standard of care in those men receiving testosterone treatment making them more likely to receive appropriate antihypertensive treatment and therefore have a lower risk of hypertension-related complications compared with men with KS who may not be receiving appropriate medical care. However, these speculations need to be confirmed through studies assessing these outcomes in association with variations in care practices.

4.4 | Thrombosis

Men with KS are challenged by a fourfold increased risk of venous thromboembolism and around 20% of all males with KS will the age of 80 years suffer from venous thromboembolism (Chang, Christiansen, et al., 2019; Zoller, Ji, Sundquist, & Sundquist, 2016), while rates of ischemic stroke and myocardial infarction are less pronounced compared with the background population (Chang, Christiansen, et al., 2019; Gravholt et al., 2018). Testosterone supplementation has been associated with an increased risk of cardiovascular disease in the general population, although the data supporting this have been much criticized (Kloner, Carson 3rd, Dobs, Kopecky, & Mohler 3rd., 2016). Available studies have mainly focused on the risk of myocardial infarction in the setting of testosterone supplementation, but registry data from UK have also indicated a potential increased risk of venous thromboembolisms 3–6 months after initiation of testosterone treatment in hypogonadal men (Martinez et al., 2016).

We recently assessed the effect of testosterone treatment on thrombotic risk in KS applying data from several Danish registries, and did not find any significant effect of testosterone treatment on rates of either venous thromboembolism or arterial thrombosis

(Chang, Christiansen, et al., 2019). Despite including the entire Danish KS population and a 22-year follow-up, the study is limited by the still relatively few observed thrombotic events, but considering the excessive risk of venous thromboembolisms among men with KS we were pleased to see that testosterone treatment was not associated with an increased risk but rather an insignificant reduction in risk (Cox proportional hazard ratio for treated versus untreated KS 0.69 [0.32–1.52]). In particular, the data indicate that testosterone treatment could attenuate the risk of venous thromboembolism in early adulthood, during which the relative risk of venous thromboembolism in KS compared with the background population is most pronounced (Chang, Christiansen, et al., 2019; Zoller et al., 2016).

The pathology behind the increased risk of venous thromboembolisms in men with KS is not clear. In our recent cross-sectional study, neither untreated nor testosterone-treated men with KS presented with a hypercoagulable state compared with age-matched controls (Chang, Christiansen, et al., 2019). Also, thrombin generation, an overall marker of coagulation, was lower in the 27 testosterone treated compared with 18 untreated men with KS (Chang, Biloft, et al., 2019). We are currently analyzing data from the same study, but also in a longitudinal setup, regarding effects of testosterone treatment in KS on fibrinolysis and platelet aggregation.

Erythrocytosis and elevation of hematocrit is well-known side effect of testosterone treatment that might in particular be associated with use of injectable testosterone (Calof et al., 2005; Jones Jr., Dukovac, Sangkum, Yafi, & Hellstrom, 2015). There is no clear evidence that secondary erythrocytosis is associated with a substantial increased risk of thrombosis (Bhatt, 2014), but emerging evidence suggests that blood viscosity and specific aspects of red blood cell function might be contributing to the pathophysiology of both venous thromboembolism and arterial thrombosis (Byrnes & Wolberg, 2017). However, in our recent cohort study we were able to assess laboratory data related to incidence of thrombosis in men with KS (Chang, Christiansen, et al., 2019). Among 19 men considered to be receiving testosterone at the time of thrombosis, only two presented with hematocrit above normal range.

The role of estrogen in mediating thrombotic risk is well described (Artero, Tarin, & Cano, 2012) and as such the relatively high estrogen to testosterone ratio in KS (Santi, De Vincentis, Scaltriti, & Rochira, 2019) and aromatization of exogenous testosterone in treated KS could increase thrombotic risk. There are, however, no available studies relating estrogen levels and thrombotic risk in men with KS.

To our knowledge, no studies evaluating thrombotic risk in boys or adolescents with KS have been conducted. Treatment with androgens does not typically lead to polycythemia in this population. With or without testosterone treatment, thrombosis in boys and adolescents with KS is rare and in our anecdotal experience usually provoked (central line, orthopedic surgery, or other high risk circumstance). While additional studies with large samples sizes are needed, there is no clear evidence suggesting that testosterone treatment should be withheld in hypogonadal individuals due to the risk of thrombosis.

4.5 | Bone metabolism

The risk of fractures and osteoporosis is increased in KS (Bojesen, Kristensen, et al., 2006), as is mortality due to fractures (Swerdlow, Higgins, Schoemaker, Wright, & Jacobs, 2005). Hypogonadism is in itself linked to reduced bone mineral density, thus leading to an increased risk of fractures and osteoporosis. Several cross-sectional studies have documented decreased bone mineral density among men with KS in comparison with controls during a broad age range (Bojesen et al., 2011; Ferlin et al., 2011; van den Bergh et al., 2001). We conducted a cross-sectional study using high-resolution peripheral Quantitative Computed Tomography scanning of tibia and radius enabling a view of the microarchitecture of both trabecular and cortical bone. We found significantly lower cortical area, total and trabecular volumetric bone mass density (BMD), trabecular bone volume fraction, trabecular number, and furthermore estimates of bone strength were also reduced at the level of the tibia. In addition, we found reduced cortical thickness at both the radius and tibia (Shanbhogue, Hansen, Jorgensen, Brixen, & Gravholt, 2014). These changes are similar to the changes seen in bone of postmenopausal women. We were not able to fully evaluate the effects of testosterone supplementation, but found no significant differences between those that had been treated and those that had not. In contrast, one study of 24 boys and adolescents with KS found bone mineral density assessed by dual-energy X-ray absorptiometry was within the normal range, with treated adolescents having a higher but not statistically significant difference in bone density (Aksglaede, Molgaard, Skakkebaek, & Juul, 2008). Other pediatric studies in KS have not evaluated bone density, but epiphyseal maturation is also an important endpoint for boys who are still growing. Boys treated with oxandrolone experienced advanced bone age even with safety stopping and dose-reduction criteria in place (Davis et al., 2017).

An uncontrolled longitudinal study of the effect of testosterone supplementation, evaluating different groups of hypogonadal patients (all hypogonadal patients, $n = 72$; KS males, $n = 21$), concluded that treatment did indeed increase bone mineral density over time (Behre, Kliesch, Leifke, Link, & Nieschlag, 1997), while another observational study concluded that only KS males below the age of 20 years of age ($n = 10$) showed an improvement in BMD, while a group of KS males above 20 ($n = 11$) did not show an improvement during an average treatment period of 5 years (Kubler, Schulz, Cordes, Beyer, & Krause, 1992). Vitamin D is important for bone and two studies have suggested that Vitamin D is lower among males with KS when compared to healthy control groups (Bojesen et al., 2011; Ferlin et al., 2015), and in an uncontrolled setting treatment with Vitamin D and testosterone replacement therapy ($n = 14$) showed an improvement in BMD over a 24-month period, while those that only received testosterone replacement therapy ($n = 12$) did not present with an improvement (Ferlin et al., 2015).

Suffice to say, there is need for much larger studies of males with KS of all ages to conclude on the most appropriate testosterone replacement therapy and supporting medication, such as Vitamin D, and whether a window of opportunity exists for initiation of therapy. In addition, there are no formal placebo-controlled studies, or studies

solely observing males with KS, that have shown that testosterone supplementation reduces fracture risk, the occurrence of osteoporosis, and improves the quality of bone in KS.

4.6 | Testicular function

Treatment with exogenous testosterone suppresses the endogenous hypothalamic–pituitary–gonadal axis in post-pubertal males. Spermatogenesis requires high intratesticular testosterone stimulated by LH, therefore there is concern that testosterone treatment may reduce the success of fertility treatments in individuals with KS. The literature in this area is mixed, with no conclusive evidence that testosterone treatment reduces sperm retrieval rates associated with testicular sperm extraction. See Corona et al. (2017) for a meta-analysis on testicular sperm extraction in KS.

In prepubertal boys, exogenous sex hormones may prematurely lift the breaks on the otherwise quiescent hypothalamic–pituitary–gonadal axis, potentially triggering early activation as seen in some conditions with chronic sex steroid exposure such as congenital adrenal hyperplasia. Although the oxandrolone protocol in prepubertal boys with KS tried to replicate normal physiology with consistent low-dose androgen exposure, nearly 25% of the boys <9 years of age receiving treatment experienced precocious puberty as determined by testicular enlargement (Davis et al., 2018). However, gonadotropins and testicular function hormones (Inhibin B, testosterone, and anti-Müllerian hormone) were not significantly different between treated and untreated groups when adjusted for pubertal status. Future intervention studies should carefully assess the effect of testosterone on testicular function and the hypothalamic–pituitary axis in both prepubertal and postpubertal boys.

4.7 | Prostatic cancer

As exemplified by a recent case presentation of prostatic cancer in a testosterone-treated man with KS (Nishikawa, Jia, Dharamshi, Charron, & Lock, 2019), there has been concern that treatment could increase the risk of prostatic cancer in men with KS. Only very few cases of prostatic cancer have been reported in KS (Nishikawa et al., 2019) and available epidemiological evidence find that the rate of prostatic cancer in KS in fact seem to be reduced and that the overall rate of cancer in KS seems to be equal to men in the background population (Bojesen, Juul, et al., 2006; Ji, Zoller, Sundquist, & Sundquist, 2016). As such there is no solid evidence suggesting that normalization of testosterone levels in men with KS would increase the risk of prostatic cancer above the rates seen among non-KS men.

4.8 | Quality of life

Although medical outcomes for patients with KS have been researched to some extent, the impact of living with KS has only been

investigated sparsely. According to World Health Organization, quality of life is defined as an individual's perception of their position in life in the context of the cultural values in which they live in addition to their goals and expectations including physical health, psychological state, level of independence, social relationships as well as personal and environmental aspects (WHO, 1995). The psychological, cognitive and social problems in addition to the medical comorbidity and sexual dysfunction associated with KS may compromise the quality of life of KS patients. In a previously published study, 75% of KS participants answered that the syndrome had significant negative consequence on their life. The greatest challenge faced by adolescents and adults with KS were infertility and psychological comorbidity, in addition to learning disabilities, physical phenotype, social relationships, employment problems, challenges with health care providers, and testosterone treatment challenges (Turriff, Macnamara, Levy, & Biesecker, 2017).

A review of the current KS literature yielded only a few studies which have focused on the impact of living with KS (Close, Fennoy, Smaldone, & Reame, 2015; de Ronde, de Haan, & Drent, 2009; Fjermestad & Stokke, 2018; Herlihy, McLachlan, et al., 2011; Skakkebaek, Moore, Chang, Fedder, & Gravholt, 2018; Turriff, Levy, & Biesecker, 2015). Overall, these studies unequivocally reported decreased quality of life or aspects of quality of life in boys, adolescents and adults with KS. In total, six studies (Close et al., 2015; de Ronde et al., 2009; Fjermestad & Stokke, 2018; Herlihy, McLachlan, et al., 2011; Skakkebaek et al., 2018; Turriff et al., 2015) have compared quality of life or aspects of quality of life of KS patients with existing data from a population normative data, a male reference group, a male control group, or without a comparison group (Table 1). The majority of studies using specific quality of life questionnaires (i.e., RAND Corporation's Short Form Health Survey [SF-36] and The Pediatric Quality of Life Inventory) found that having KS affected physical aspects, social function, emotional health, mental health and general health in childhood (Close et al., 2015), adolescence (Close et al., 2015), and adulthood (de Ronde et al., 2009; Fjermestad & Stokke, 2018; Skakkebaek et al., 2018).

To further understand the underlying cause of the decrement in quality of life seen in boys and men with KS, some of the studies investigated several variables that might be important predictors of quality of life. In two of the studies, phenotypic severity was demonstrated to affect quality of life. In the study by Close et al., a combined index of (total) quality of life, physical quality of life, and social quality of life were found to correlate negatively with phenotypic severity with 22 % of the variance in total quality of life explained by the phenotype (Close et al., 2015). Herlihy, McLachlan, et al. (2011) also reported phenotypic severity as the strongest predictors of all outcomes in adults with KS, although employment status and social support also influenced quality of life. In contrast to phenotypic severity, higher level of education were found to correlate positively with quality of life (3), whereas poor sleep affected quality of life negatively (Fjermestad & Stokke, 2018). In our recently published study, we evaluated predictors of mental quality of life as well as physical quality of life. Our models yielded that lower mental quality of life in patients with KS was associated with lower income and living without a

partner, whereas a correlation between lower physical quality of life with less employment, lower income, daily medicine intake, and less physical activity were seen (Skakkebaek et al., 2018). In addition, we found evidence that having KS in itself is associated with poorer life quality.

The impact of testosterone treatment on quality of life in patients with KS has not been investigated sufficiently. However, the current literature does not support an impact of testosterone on quality of life. In the study by Close et al. no association between quality of life and total testosterone level in blood were seen (Chang et al., 2015). In support, we did not find any significant differences in quality of life scores between treated and nontreated adults in our Danish men with KS (Skakkebaek et al., 2018). These results, however, do not match with our clinical impression, where we see a clear benefit in most adult KS patients from testosterone therapy with improvement in social functioning, sexual function, sleep, and vigor. Also, there are indications of a positive effect of early testosterone supplementation on improving the behavioral phenotype in boys with KS (Samango-Sprouse et al., 2015), although these studies are purely observational and without proper randomization and inclusion of a placebo-treated control group of males with KS. There are important inherent ascertainment biases in the existing studies that may account for the lack of observing a beneficial effect of testosterone on quality of life outcomes and additional longitudinal investigation is warranted.

Although the current literature demonstrate a picture of patients with KS as suffering from poorer quality of life, and that the syndrome have severe implications for the majority of boys and men with KS, these patients seem to cope with their situation. Turriff et al. found that adolescent and adults with KS have a high level of adaptation, and were able to use different coping strategy (Turriff et al., 2015). Interventions teaching these effective coping strategies could be implemented for individuals with KS to potentially improve quality of life measures.

In conclusion, our knowledge about quality of life and aspects hereof in patients with KS is still limited. Future studies would be beneficial to further increase our knowledge about the lived experience of KS.

5 | TESTOSTERONE TREATMENT OF KS IN CLINICAL PRACTICE

5.1 | Timing of testosterone treatment in KS

Because of the lack of consensus guidelines there are not specific criteria for testosterone supplementation in KS, and perhaps most importantly when treatment should commence. Clinical practice in most countries has been that testosterone supplementation should be timed with the natural onset of puberty if testosterone production is inadequate and/or LH and follicle stimulating hormone rise above normal limits, which is the strategy that we use in our clinic (Gravholt et al., 2018). Others argue that one should not start testosterone supplementation before serum total testosterone falls below normal ranges. However, since most KS cases are diagnosed in adulthood

TABLE 1 List of studies investigating quality of life (QoL) in boys and men with Klinefelter syndrome (KS)

Study	Design	Participants	Instrument related to QoL	Results	Factors influencing QoL
De Ronde et al. (2009)	Observational design, no control group (Dutch male reference group)	40 KS (mean age: 46 ± 11.8 years)	RAND Corporation's short-form health survey (SF-36)	Significant lower scores on 8 out of 9 subscales	Education
Herlihy, Halliday, et al. (2011)	Observational, no control group (Australian population normative data)	87 KS (mean age: 43 [19–76 years])	Personal Well-being Index (PWI); global life satisfaction; Multidimensional Body-Self Relations Questionnaire—Appearance Scale; Rosenberg Self-esteem Scale; Kessler psychological distress scale (K10); self-assessed health status (short form 1); Quality of Relationships Inventory	Poorer outcomes for all measurements	Employment status, social support and phenotype
Close et al. (2015)	Cross-sectional	43 KS (mean age: 12.5 ± 3.1 years [8–18])	The pediatric quality of life inventory 4.0; Piers-Harris 2 self-concept scale; Coopersmith self-esteem inventory; Children's Depression Inventory	68% showed lower QoL, 26% had poor self-concept, 38% had low self-esteem	Phenotype
Turriff et al. (2015)	Observational	310 KS (mean age: 40.7 ± 14 years [14–75])	Psychological Adaptation Scale; Illness Perception Questionnaire; Perceived Social Stigma Scale; Ways of Coping Checklist-Revised	76.4% reported a negative consequence of KS, the majority did not perceive stigmatization, the majority reported positive adaptation to living with KS	How KS patient coped were the greatest predictor of adaptation
Traish et al. (2017)	Observational	310 KS (mean age: 40.7 ± 14 years [14–75])		Challenges of XXY, positive impact of KS	
Fjermestad and Stokke (2018)	Observational, no controls (norm data for Norwegian males, norm samples from Canada and Australia)	53 SCA (mean age: 36.8 ± 12.3 years [19–67]) (77% KS)	RAND Corporation's short-form health survey (SF-36); PWI; Pittsburgh Sleep Quality Index (PSQI)	Significant poorer scores on all subscales of SF36 and PSQI, but only small changes seen in PWI.	Sleep quality
Skakkebaek et al. (2018)	Cross-sectional	132 KS (mean age: 41.7 [19.0–76.5 years]) 313 controls (mean age: 42.5 [17.0–77.2 years])	World Health Organization's quality of life assessment (WHOQOL-BREF); RAND Corporation's short form health survey (SF-36)	Significant poorer scores on all subscales of SF36 and WHOQOL-BREF.	Mental QoL: income, living with partner Physical QoL: Employment, income, medicine intake, physical activity, sexual function, age, partner status

(Berglund et al., 2019; Garolla et al., 2018), the pubertal window for initiation of treatment is often missed, and supplementation is then initiated after a diagnosis has been made if hypogonadism is present, which it almost invariably is. We find that in Denmark, the most common reason for delay of treatment after diagnosis is fertility treatment. Testosterone supplementation lowers gonadotropins through negative feedback and has been associated with decreased sperm retrieval rates in men with KS by some (Garolla et al., 2018). However, the impact of testosterone supplementation on biological paternity in KS is still debatable, since others do not find that testosterone treatment impairs chances of fertility (Zganjar, Nangia, Sokol, Ryabets, & Samplaski, 2019). In recent years there has been a growing focus on potential beneficial effects of testosterone supplementation in prepubertal boys (Davis et al., 2017; Ross et al., 2017). In a recently published survey among U.S.-based adult endocrinologists ($n = 133$), pediatric endocrinologists ($n = 60$), and urologists ($n = 39$), 15% responders stated that infants with KS should be started on testosterone supplementation, and 42% of responders including the majority of pediatric endocrinologists stated that infants with KS should be started on testosterone supplementation in the presence of a micropenis (Zganjar et al., 2019). Two-thirds of responders agreed that the optimal timing for initiation of testosterone supplementation in KS was between 10 and 16 years of age, although there was no real consensus on whether to monitor testosterone levels, gonadotropin levels, or to look for clinical signs of delayed puberty or hypogonadism, to decide the actual timing of initiating treatment in the individual patient (Zganjar et al., 2019). Nobody discussed issues such as a probable effect of testosterone supplementation on learning ability and betterment of neurocognitive abilities. Several studies, investigating various health-related outcomes in boys with KS treated with testosterone are under way and will provide more insight to the potentially benefits of early treatment in KS (ClinicalTrials.gov identifier: NCT02408445, NCT00348946, NCT02723305, NCT03325647).

5.2 | Testosterone treatment of KS in Denmark

Citizens in Denmark have equal access to public funded primary physicians and hospitals. However, most prescription medications, including testosterone preparations for KS, are subject to own payment, but with a considerable public subsidy. The annual expenses for testosterone supplementation for a man with KS under these circumstances are currently around 2000 DKR (≈ 300 USD; 270 EURO). Care of KS in Denmark has in recent years been centralized at specialized endocrinology clinics at University Hospitals to offer care of males with KS across the entire lifetime. In the specialized centers all relevant medical specialties are represented and there is also a procedure for securing transition from pediatric to adult care. We are confident that implementation of centralized interdisciplinary clinics not only improves overall care of men with KS, but could also be a means for addressing the diagnostic deficits by broadening awareness of KS and the nonendocrinological symptoms among a wider set of health care professionals. Similar approaches are being implemented outside of

Denmark (Salzano et al., 2016; Tartaglia et al., 2015) with the establishment of specialized KS clinics for both boys (Tartaglia et al., 2015) and men (Kalia, 2019) with KS.

There are very few data regarding testosterone treatment in KS. However, recently we published nationwide data illustrating the use of testosterone among men with KS in Denmark (Chang, Christiansen, et al., 2019). We included all men with a known KS karyotype living in Denmark between 1994 and 2016 ($n = 1,115$) and collected prescription data during the same period from the Danish National Prescription Registry. We demonstrated that only 48.7% ($n = 563$) of men with KS had one or more redeemed prescriptions for testosterone. The median interquartile range (IQR) age at diagnosis among men with KS later treated with testosterone was 25.5 (15.3–34.3) years, but the median interquartile range (IQR) age at first testosterone prescription was 30.4 (19.2–40.9) years, indicating a delay of treatment of around 5 years. Reasons for this delay were not assessable in the data, but could be due to fertility treatment, absence of overt hypogonadism at diagnosis, or patient-centered reasons for declining treatment. However, in our experience, most adult men with KS have overt hypogonadism at presentation and further, by including laboratory data from Danish hospitals, we were able to demonstrate biochemical signs of hypogonadism in those men with KS without testosterone prescriptions (Chang, Christiansen, et al., 2019). We consider it surprising that there is such a low rate of treatment in the entire Danish cohort of men with KS and such a relatively long delay before initiation of treatment. Indeed, in a recent ongoing survey of patients at our clinic ($n = 160$), all, except very few ($n = 3$), were receiving appropriate testosterone substitution therapy (unpublished observation). This suggests that the delay in treatment is more likely due to failure of providers outside these specialized centers to recommend appropriate treatment and could indicate a lapse of care of men with KS, even in a health care system as accessible as the Danish system. Of course, nontreatment could be a personal preference, due to, for instance, a lack of disease awareness or fears of adverse effects. However, in our clinical experience very few men with KS and hypogonadism will actively opt to not receive testosterone treatment if offered and thoroughly explained reasons for treatment. On the upside, we did find that those men with KS receiving testosterone treatment were on average born and diagnosed more recently compared with the untreated group, which could indicate an improving standard of care over the last years (Chang, Christiansen, et al., 2019) (Figure 1).

Among those treated with testosterone, 16% ($n = 89$) had prescription for transdermal testosterone only, 46% ($n = 258$) had prescription for injectable testosterone only, 31% ($n = 174$) had prescription for both transdermal and injectable testosterone, and 7% ($n = 42$) had prescription for oral testosterone only (Chang, Christiansen, et al., 2019). Since treatment will likely be lifelong, we at our clinic encourage patients to use both transdermal and injectable formulations for a period of at least 6 months to find the one treatment best suited for them. Further studies are needed to evaluate which route of treatment should be preferred in relation to relieving signs of hypogonadism, maximizing patient adherence to treatment and minimizing the risk of adverse effects.

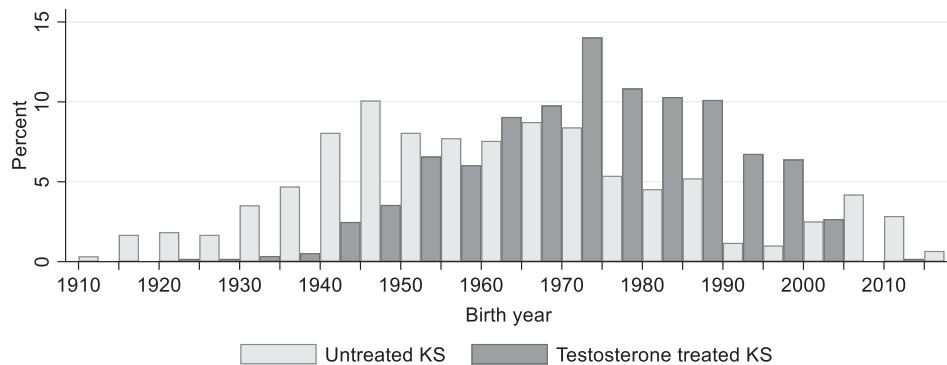


FIGURE 1 Distribution of birth year in untreated and testosterone-treated Klinefelter syndrome (KS) in Denmark. The men in the cohort of untreated KS are either individuals that have not yet reached puberty or individuals that are on average born 12 years earlier than the testosterone-treated cohort. This could indicate that awareness of the need for testosterone treatment in KS has improved over the years. Reproduced with permission from Chang, Christiansen, et al. (2019)

6 | SUMMARY AND CONCLUSIONS

KS is the most common sex chromosome abnormality in males, but often overlooked. It is becoming increasingly clear that KS affects all aspects of life and that treatment of KS is demanding a multidisciplinary approach. Hypogonadism is virtually omnipresent in adult KS making testosterone treatment the cornerstone of care for men with KS, but the available data suggest that a large proportion of patients are likely not being offered relevant treatment.

There are few longitudinal or randomized studies investigating health-related outcomes of testosterone treatment in KS, but the available evidence stemming from mostly cross-sectional studies indicate beneficial effects of testosterone treatment on factors contributing to the excess morbidity burden and mortality seen in adult KS. Long-term data on endpoints such as mortality, morbidity, neurocognitive function, quality of life, and socioeconomic traits are warranted. Of notice, the available studies so far have not identified serious adverse events associated with proper managed testosterone treatment in adult KS, which reassuringly suggests that testosterone treatment in men with KS is safe.

To provide optimal care for boys and men with KS, it is required to establish specialized multidisciplinary clinics include a formalized structure ensuring seamless transition from pediatric to adult care. Centralization of care would likely also benefit KS research by simplifying both participant recruitment and the process of setting up larger international multicenter studies. Performing long-term placebo-controlled studies on testosterone treatment in truly hypogonadal men with KS presents with several ethical considerations and recruitment of participants could also be challenging. An international collaborative study, investigating a broad array of outcomes could be a way forward. Such studies are needed to address issues such as timing of testosterone supplementation, different modalities of treatment and how to best monitor treatment and would be of high value in creating an evidence and consensus based guideline for care in KS.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

ORCID

Simon Chang  <https://orcid.org/0000-0003-1130-3659>

Anne Skakkebaek  <https://orcid.org/0000-0001-9178-4901>

Shanlee M Davis  <https://orcid.org/0000-0002-0304-9550>

Claus H Gravholt  <https://orcid.org/0000-0001-5924-1720>

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