Klinefelter Syndrome: Integrating Genetics, Neuropsychology, and Endocrinology

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ABSTRACT Although first identified over 70 years ago, Klinefelter syndrome (KS) continues to pose substantial diagnostic challenges, as many patients are still misdiagnosed, or remain undiagnosed. In fact, as few as 25% of patients with KS are accurately diagnosed and most of these diagnoses are not made until adulthood. Classic characteristics of KS include small testes, infertility, hypergonadothropic hypogonadism, and cognitive impairment. However, the pathophysiology behind KS is not well understood, although genetic effects are also thought to play a role. For example, recent developments in genetics and genomics point to a fundamental change in our understanding of KS, with global epigenetic and RNA expression changes playing a central role for the phenotype. KS is also associated with more general health markers, including higher morbidity and mortality rates and lower socioeconomic status (which likely affect both morbidity and mortality). In addition, hypogonadism is associated with greater risk of metabolic syndrome, type 2 diabetes, cardiovascular disease, breast cancer, and extragonadal germ cell tumors. Medical treatment typically focuses on testosterone replacement therapy (TRT), although the effects of this therapy have not been studied rigorously, and future studies need to evaluate the effects of TRT on metabolic risk and neurocognitive outcomes. This review presents a comprehensive interdisciplinary examination of recent developments in genetic, endocrine, and neurocognitive science, including the study of animal models. It provides a number of recommendations for improving the effectiveness of research and clinical practice, including neonatal KS screening programs, and a multidisciplinary approach to KS treatment from childhood until senescence. ***(Endocrine Reviews 39: 389 – 423, 2018)***

Klinefelter syndrome (KS), 47,XXY, occurs in 150 per 100,000 live born males (1). No universal agreement exists in the scientific community on the exact definition of KS, but in addition to possessing one or more extra X chromosomes, KS males typically exhibit phenotypical traits that include hypergonadotropic hypogonadism, testosterone deficiency, and infertility (2). Phenotypic variability, however, often leads to diagnostic delay or nondiagnosis, with an estimated 50% to 75% of males with KS never obtaining correct diagnosis (1, 3). KS can have profound adverse consequences, as morbidity and mortality among known KS males are significantly higher than in the general population, and these health risks are presumably even higher among those KS males who never receive testosterone replacement therapy (TRT) by virtue of not being correctly diagnosed (4–9). The key to more timely diagnosis and treatment of KS is a more comprehensive understanding of its etiology, characteristics, and effects, which is the goal of this review.

Risk assessment in KS is compromised by insufficient insight into the prevalence and causes of different syndrome-associated traits that may impact adversely on prognosis. This is especially the case for genetic, endocrine, cardiovascular, neurocognitive, and behavioral contributions to the wide range of diseases, which together contribute to excess all-cause

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ESSENTIAL POINTS

- Identification of Klinefelter syndrome (KS) is more difficult than previously anticipated, with only a fraction (25% to 40%) of cases accurately diagnosed
- KS is associated with a globally changed DNA methylation profile, with large areas of hypermethylation and, to a lesser degree, hypomethylation
- Morbidity and mortality is increased from a wide variety of diagnoses, many that are not easily reconciled to the known KS phenotype or effects of hypergonadotropic hypogonadism
- Although hypogonadism is among the classic characteristics of KS, the effects of testosterone replacement therapy are not well studied, and many questions concerning timing, dose, and route of administration remain to be answered
- Individuals with KS also experience pervasive neurocognitive deficits, which pose additional challenges for these patients
- Multidisciplinary clinics should be the mainstay throughout the world in clinics treating those with KS

mortality (10). Because late diagnosis and nondiagnosis is frequent, ascertainment bias may obscure the epidemiological picture of many KS aspects, and current attempts at providing guidelines may well underestimate both morbidity and mortality. Previous guidelines and reviews have all relied on expert consensus and have not included a broad base of professionals working with KS (2, 11–13). This is unfortunate because complex patterns of endocrine, psychiatric, and other diseases in KS render direct translation of evidence from other cohorts hazardous. More educated risk stratification and more appropriate clinical care can only be facilitated through a thorough delineation of the phenotype in KS.

This review provides specific insights into the genetic, endocrine, metabolic, cardiovascular, and neurocognitive phenotype in males with KS and presents an up-to-date synopsis of the latest body of knowledge, emphasizing the significance of both congenital and acquired pathologies. The aim is to provide an update on current insight into the pathogenesis of KS and relate to recent advances in the understanding of the dosage effect of having an extra X chromosome, exposure to X inactivation, and its influence on male health. Moreover, this review provides an updated hypothesis on the genetic etiology of KS, highlighting our knowledge about importance of X chromosomal aneuploidy to congenital and acquired neurocognitive and endocrine traits. Finally, this review incorporates important endocrine features of KS, accounting for how genetics may explain the prevailing phenotype and how attention to endocrine factors is important in efforts to identify and modify risk markers. Where available, data from animal models are included. We conclude with perspectives on where science may take us in the future.

The full PubMed database was searched (without time restrictions) in May 2017 using the keyword "Klinefelter syndrome" as the Medical Subject Heading(MeSH) term, as well as "Klinefelter syndrome," "Klinefelter's syndrome," "Klinefelter," and "Klinefelter's" in titles and abstracts. Articles relevant to the individual topics were obtained and reviewed, as well as older articles selected by the authors. Publications cited in this review were selected from those identified by the searches at the authors' discretion.

Prevalence, Morbidity, and Mortality

When KS was first described by Harry F. Klinefelter, Edward C. Reifenstein, and Fuller Albright in Boston in 1942 (14), the authors described the occurrence of the syndrome as "not uncommon." When Patricia A. Jacobs and John A. Strong in 1959 (15) described the karyotype 47,XXY, it became possible to verify the diagnosis by standard karyotyping and thus to examine large populations. However, although many surveys of newborns subsequently have been performed, it is still not clear how frequent KS is, and especially whether there are demographic differences in prevalence. It is nonetheless clear that diagnosis and especially late or nondiagnosis of the syndrome is of substantial concern. Few boys with KS are diagnosed, and only a minority of the expected number is diagnosed during adulthood (1). Nondiagnosis may likely introduce ascertainment bias and hamper the interpretation of the current literature on KS. Are nondiagnosed KS males similar to diagnosed KS males? Are they much less affected with no or few symptoms, which could explain the conundrum of nondiagnosis, or are they more severely affected leading to premature death before diagnosis? In this section, all aspects of epidemiology will be discussed, and areas of uncertainty will be highlighted.

Prevalence

Based on a number of large cytogenetic chromosome surveys of newborns in various countries around the world, it is possible to compute an estimate of the average prevalence of KS at birth, which is 152 per 100,000 newborn males [95% confidence interval (CI),

121 to 188 per 100,000] (a total of 84 diagnosed with KS in 55,212 boys), ranging from 85 to 223 per 100,000 males (16–23). Still, these studies were all performed primarily with white and Japanese individuals. More recent research in Denmark, Australia, and the United States (3, 24, 25) has replicated these results, along with showing a significant diagnostic divergence dependent on the time of diagnosis. In Denmark, we determined the prevalence of KS based on prenatal examination to be 153 (145 to 161) per 100,000 liveborn males, which is very close to previous estimates (1), and because spontaneous abortions rarely occur (24), this prevalence can be seen as a valid index of prevalence in Denmark. The prevalence based on postnatal examination, however, was much lower, and for the entire study period from 1931 to 2000, only 28 KS males per 100,000 were detected, which illustrates low diagnostic yield in the beginning of the 20th century. But even so, it is clear that many KS males are not diagnosed, and we estimated that only about 25% of all KS males were diagnosed postnatally. In a study from Australia, a somewhat higher pre- and postnatal prevalence of 223 (195 to 254) per 100,000 and 87 (70 to 107) per 100,000 was presented (3), indicating that about 40% of the expected KS males were diagnosed postnatally. The authors speculate that the higher pre- and postnatal prevalence in Australia compared with Denmark may be due to the combined effect of older Australian mothers and a different racial composition of the Australian populace. A recent study from the United States suggested that the prevalence of KS among males with white ethnicity was 166 per 100,000, but 355 per 100,000 among males with Asian ethnicity, although numbers in this study were small (25).

Diagnosis and nondiagnosis

The diagnosis of a male with KS rests on clinical appearance coupled with a karyotype of 47,XXY or mosaics thereof. There is no universal agreement on the necessary clinical signs or stigmata that should lead to karyotyping (2, 26). We believe that persons with additional sex chromosomes (48,XXXY, 48,XXYY, and other similar syndromes) should not be considered KS males, because they normally have a much more affected phenotype (27). The cardinal stigmata include small testes (which are present in virtually all KS males), hypergonadotropic hypogonadism, gynecomastia, learning difficulties, and infertility. It is clear though that many other signs, symptoms, and conditions can be associated with KS (Table 1). However, absence of overt clinical signs is often the case, and many males with KS are difficult to distinguish from the normal 46,XY male (29, 30). As mentioned previously, epidemiological studies have estimated the diagnostic yield in different countries. It seems that only 25% to 40% of the pool of KS males are ever diagnosed (1, 3, 51), and only about 10% of these are diagnosed during childhood and adolescent years, whereas the bulk of patients are diagnosed during adulthood, typically in the course of a fertility workup (3), as shown in these updated Danish data (Fig. 1), which found a mean age at diagnosis of 27 years. It can be seen from these new data that 65% of prenatally diagnosed KS males are legally aborted, that only a small fraction are diagnosed in childhood, and that a minority are diagnosed quite late in life after the age of 50 years. A British study on mortality in KS males found 3518 individuals with KS ever diagnosed by the year 2000, and one can indirectly make a crude estimate of the prevalence to 11.9 per 100,000 males [UK population (2000): 58.89 million, estimated male population: 29.45 million] (4). Likewise, a recent Swedish study on cancer epidemiology found 1085 individuals with KS, and one can estimate a prevalence of 23.1 per 100,000 males [Swedish male population (2010): 4.69 million] (6), which shows that far fewer KS males are diagnosed in Great Britain and Sweden than would be expected. These crude estimates do not adjust for the somewhat elevated mortality rate that is present among KS males (4, 5, 8, 10), but even so, they illustrate that the diagnostic yield maximally reaches 40% of the expected number in all countries with available nationwide data. These figures beg the question of why so many KS males are not diagnosed. KS can be diagnosed prenatally by amniocentesis, chrorion villus sampling, or cell-free DNA testing (52). Furthermore, Down syndrome screening using ultrasound-based nuchal fold measurement, serum pregnancy-associated protein A, and free β human chorionic gonadotropin detects 19 KS males per 100,000 males fetuses (13% of the expected number) (24). Evaluation of all available studies from a wide range of countries shows that about 44% to 85% of parents choose legal abortion of a KS fetus (24, 53), reducing the number of liveborn KS males, but only marginally due to the low level of detection of KS by prenatal methodology. Applying the legal abortion rate to KS prevalence found in our previous studies, legal abortion in Denmark at the present would be expected to reduce the prevalence of liveborn KS males from 150 to 140 per 100,000 males (1, 24). This may well change in the future with optimization of especially cell-free DNA testing leading to detection of much greater numbers of KS males, given parents will continue to choose legal abortion with a rate of 45% to 85% (24, 53). Taken together, available data show that diagnosis of KS is often seriously delayed, and frequently a diagnosis is never made (51), illustrating that new diagnostic avenues should be implemented. Late diagnosis or nondiagnosis extends to all sex chromosome syndromes (54–57). We, and others, have called for population-based, neonatal genetic screening to clarify several questions concerning prevalence and phenotypic spectrum and enabling early establishment of appropriate treatment (58–60). Population-based, neonatal screening can be considered if a condition

Table 1. Abnormalities and Diseases Associated With KS

Feature	Frequency (%)
Adults	
Infertility (28)	>99
Azoospermia (28)	>95
Decreased bitesticular testis volume (4–8 mL; normal range: 25–60 mL) (29–31)	>95
Sperm after TESE	30–50
Decreased beard growth[a] (28)	60–80
Decreased pubic hair[a] (28)	30–60
Abdominal adiposity (32)	~50
Decreased muscle mass and strength (30, 33)	~40
The metabolic syndrome (32)	46
Type 2 diabetes (32)	10–39
Osteopenia (33, 34)	~40
Osteoporosis (33, 34)	5–10
Mitral valve prolapse (35, 36)	0–50
Ischemic heart disease (8, 9)	~1.5-fold increased risk[b]
DVT and PE (4, 7, 9)	Three- to sixfold increased risk[b]
Autoimmunity (37–39)	Increased risk of several autoimmune diseases
Tremor (Parkinson-like symptoms) (40, 41)	>25
Breast cancer (5, 6, 42)	Increased risk (approximately fourfold[b])
Osteoarthritis (9)	Fourfold increased risk[b]
Children	
Learning disability (18)	>75
Delayed speech development (18)	>40
Decreased penile size (18, 43, 44)	10–25
Mediastinal cancer (45)	
All patients with KS	
Gynecomastia (18, 30, 46)	28–75
Cryptorchidism (9, 18, 29)	27–37
Increased gonadotropin levels[a] (28, 29, 31)	>75
Decreased testosterone levels[a] (28, 29, 31)	>75
Increased height (18, 30)	>30
Psychiatric disturbances (18, 47)	>25
Congenital malformations (heart, cleft palate, and inguinal hernia) (4, 9, 48)	Increased risk
Fractures (4, 9)	Increased risk (2- to 40-fold)[b]
Autism spectrum disorder (49, 50)	30–50

[a] In the untreated condition.
[b] Above-normal male frequency.

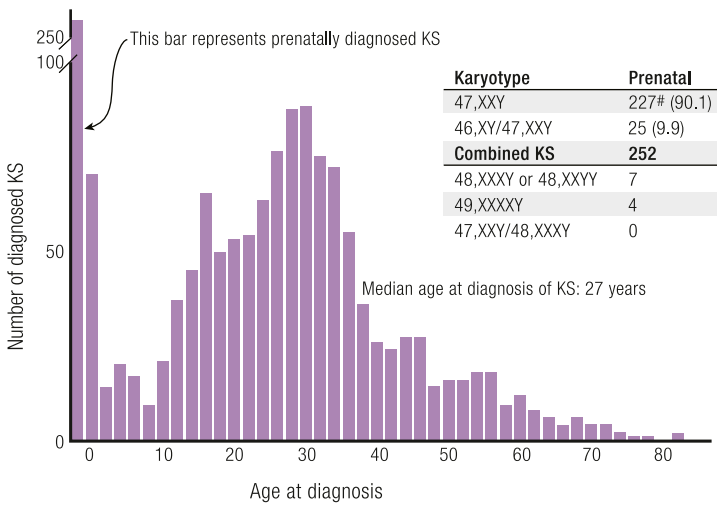
is an important health problem with a latent, early asymptomatic stage and has a well-understood natural history for which there are accepted treatments with associated facilities for diagnoses and treatment (61). We think that these requirements are fulfilled for KS, although due to the rarity of the syndrome, it will likely take a long time to demonstrate associations between early diagnosis, continuous specialized care, and improved long-term outcomes.

Morbidity

The morbidity pattern among KS males is diverse (Fig. 2), which is difficult to reconcile with the different phenotypic characteristics usually present among KS males, including hypergonadotrophic hypogonadism, infertility, and neurocognitive deficits. We investigated the Danish registries regarding the morbidity pattern in KS and found an elevated morbidity for almost all International Classification of Diseases and Related Health Problems, version 10 (ICD-10) chapters (except the chapter "Diseases in the Newborn") (9). Infections, certain cancers (breast and mediastinal), anemia, psychiatric diseases (psychoses, neuroses/personality disorders, mental retardation), neurologic diseases, circulatory diseases [ischemic heart disease, deep vein thrombosis (DTE), lung embolia] (7), pulmonary diseases (pneumonia, chronic obstructive pulmonary disease, asthma), gastrointestinal disease (ulcus, cirrhosis of liver), skin diseases, diseases of the musculoskeletal system (osteoarthritis), diseases of the urogenital system (infections, gynecomastia), congenital malformations (heart, genitalia, retention of the testis), trauma, and intoxications were all seen significantly more among KS males. Endocrine diseases were as well seen more frequently among KS males, including type 1 diabetes, type 2 diabetes, hypogonadism, and hypothyroidism. At that time, some of these diseases had previously been associated with KS, but many had not. This applied to conditions like pneumonia, chronic obstructive pulmonary disease, asthma, osteoarthritis, ulcus, *etc.*, likely due to their commonality in the general population.

The pattern of malignant disease in KS shows that although the risk of cancer in general is close to that of the normal male population (5, 6, 9), certain patterns emerge with a higher risk of breast cancer, mediastinal tumor, non-Hodgkin lymphoma, and hematological cancers in general, whereas the risk of prostate cancer is low. It remains unexplained why some cancers are more frequent among KS males, but a genetic background seems plausible. On the other hand, it is likely that the scarcity of prostate cancers is due to low levels of endogenous testosterone in the untreated condition, and/or undertreatment with testosterone, and thus relative hypogonadism among many KS males. A recent large study of non-KS males in the United Kingdom pointed toward increased body fat and presence of diabetes as protective risk markers (62), which also could be at play in KS.

Such a pattern of morbidity points to factors other than just hypogonadism, genetic causes, and decreased neurocognitive deficits as explanatory factors, and we have extended our register studies and included socioeconomic variables accordingly. In a recent study, we show that the socioeconomic status of KS males is very different from that of controls. Less than 10% of KS males achieve a higher education, whereas retirement age on average is more than 15 years earlier



Karyotype	Prenatal	Aborted	Postnatal	Born†
47,XXY	227# (90.1)	153 (93.3)	1135* (90.7)	1209 (88.6)
46,XY/47,XXY	25 (9.9)	11 (6.7)	117 (9.3)	156 (11.4)
Combined KS	**252**	**164 (65.1)**	**1252**	**1365**
48,XXXY or 48,XXYY	7	7 (100)	45	45
49,XXXXY	4	4 (100)	16	16
47,XXY/48,XXXY	0	0	8	8

Figure 1. Age at diagnosis of 252 and 1252 prenatally and postnatally diagnosed males with KS in Denmark by 31 December 2015. For comparison, diagnosed persons with additional sex chromosomes are included in the table. Based on data from Bojesen *et al.* (1). The first bar (arrow) indicates the number of prenatally ascertained cases. Inset table: in parentheses, percentages are provided. †The category "born" includes all postnatal ascertained KS males and all prenatal ascertained, and not legally aborted, KS males (n = 88). Some prenatally ascertained cases (n = 57) were tested both prenatally and postnatally, whereas the remaining (n = 31) were only tested prenatally. #Including two cases of 48,XXY,+18, one case of 48,XXY,+21, and one case of 48,XXY,+16. *Including five cases of 48,XXY,+18 and two cases of 48,XXY,+21.

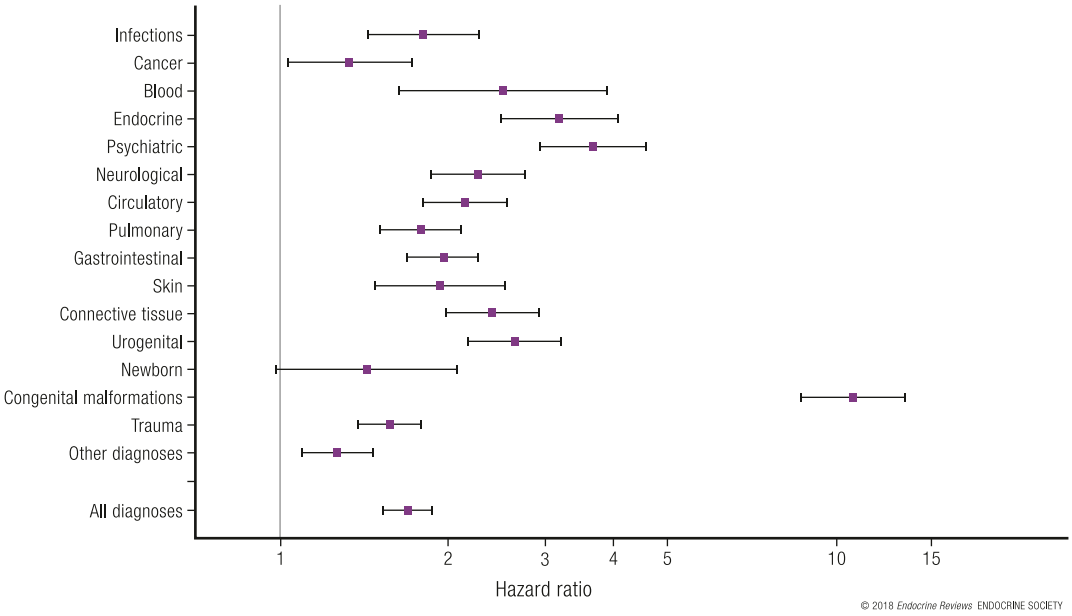


Figure 2. HRs with 95% CIs for ICD-10 diagnostics groups and for all diagnoses combined. Reprinted with permission from Bojesen *et al.* (9).

(KS males vs. controls: 43.5 vs. 60.3 years). Fewer KS males become fathers, and more live alone (10). As a result, the annual income throughout the lifespan is much lower among KS males. The reduced socioeconomic status that emerges is undoubtedly an explanatory factor for the diverse morbidity pattern, as shown in other settings (63), but we still need to untangle the additive effects of the syndrome *per se*, late diagnosis, undertreatment of hypogonadism, and possibly also inadvertent overtreatment with exogenous testosterone.

Mortality

All studies of mortality rates among KS males have found this to be greater than among matched controls or the general population (4, 8, 10, 64). The same is seen among other sex chromosome abnormality syndromes, such as Turner syndrome (65, 66), 47,XXX (55, 67), and 47,XYY (56, 64). This seems to be an intrinsic consequence of sex chromosome aneuploidy and as such a common trait, which comparative analyses point toward (64, 67). In our latest published analysis, we have estimated that mortality among KS males is increased with a hazard ratio (HR) of 1.9, which corresponds to median loss of 5.6 years (10). The excess mortality among KS males follows the pattern from the morbidity data and is a result of a wide range of diseases, including diseases of the circulatory, respiratory, endocrine, and metabolic systems, and of cancer (Fig. 3). The increased risk of death among KS males may partially be explained by their lower socioeconomic status (10). An updated analysis of the total cohort of KS males in Denmark (Fig. 1), including all diagnosed until December 2016, shows an overall mortality HR of 1.63 (1.45 to 1.83, $P < 0.05$). Moreover, HR before 30 years of age is 4.18 (2.72 to 6.40) and HR before 15 years of age is 9.56 (4.40 to 20.76). These estimates show that KS males diagnosed at an early age have a very high mortality relative to age-matched controls. We currently do not understand the basis for this elevated mortality, but we speculate that the cohort constituting the young group of diagnosed KS males has a much more complicated phenotype, which leads to early diagnosis in the first place and thus a higher mortality.

Summary of best evidence

The prevalence of KS ranges from 85 to 250 (–355) per 100,000 liveborn males, and it is possible that there are ethnic differences. Only a minority (25% to 40%) of the expected number of KS males are ever diagnosed. Morbidity and mortality is increased across all diagnostic chapters, with likely influences from hypogonadism, genetic factors, and poor socioeconomic conditions and perhaps also from TRT.

Areas of controversy

There is a dire need for population-based studies in different ethnic groups, both to establish a valid and reliable prevalence and to assess the impact of ethnicity. It is currently not clear how to improve the diagnostic yield in the best way. We advocate for the introduction of population-based, neonatal screening, although the cost-benefit ratio of such an initiative has yet to be evaluated. It is not clear how TRT impacts the general pattern of morbidity and mortality as well as specific diseases.

Genetics of KS

Neither the origin nor the phenotypic manifestation of sex chromosome abnormalities is well-understood,

and phenotypic features consistently associated with these syndromes remain elusive (68). Other than KS, sex chromosome syndromes include Turner syndrome (45,X), 47,XXX syndrome, and 47,XYY syndrome. The current understanding of the X and Y chromosomes is based on evolutionary research; the sex chromosomes evolved from an identical pair of autosomes, but whereas the X chromosome has retained most of the original genes (649 genes), the Y chromosome retained only about 40 genes, of which 17 are shared with the X chromosome. These mutual genes are involved in regulating other genes throughout the entire genome (68). Bellott *et al.* (68) showed that 12 of the remaining genes on the Y chromosome, having identical haplotypes on the X chromosome, are needed in exactly two copies, and thus might play a vital role in sex chromosome abnormalities (1).

The overarching biological question related to sex chromosome abnormalities is how to merge the understanding of the genome, epigenome, and transcriptome, for example, with the different phenotypic manifestations related to different organs, viability, occurrence of congenital malformations, *etc*. Similarly, traits as severe and diverse as type 2 diabetes, intrauterine demise, congenital cardiovascular malformations, and altered neurocognitive performance remain largely unexplained. Very recent studies with a system biology approach suggest an increased X chromosome gene dosage linked with altered protein interactome activity as an explanation for the observed comorbidities among KS males (69). The authors have epidemiologically mined a patient registry, merged with RNA expression data and exploited protein-protein interaction databases, to discover altered Jak-STAT signaling, dysregulated genes involved in immune system function, energy balance (*POMC* and *LEP*), and erythropoietin signaling to be present in complex comorbidity networks in KS males (69).

Genotype-phenotype associations

Despite comprehensive research, our knowledge about the genotype-phenotype relation in KS is limited. Genetic mechanisms related to the X chromosome as well as the androgen receptor have been evaluated for a possible impact on the phenotype in KS. These genetic mechanisms include the parental origin of the supernumerary X chromosome, the pattern of X chromosome inactivation, and the androgen receptor CAG repeat length.

The 47,XXY karyotype arises from nondisjunction, either as a paternal nondisjunction in the first meiotic division (50% of cases) or as maternal nondisjunction in first or second meiotic division or during postzygotic division (50% of cases) (70, 71). The possibility that the parental origin of the extra X chromosome should have an impact on the phenotype has been proposed. The evidence is sparse, however, with the majority of studies finding no association (44, 72–74), and only few finding a parental origin effect on phenotypic traits, including motor function and language/speech (75), autistic and schizotypal traits (76), onset of puberty (77), and the ratio of waist and height to arm span (30).

In KS males as in females, one of the two X chromosomes is inactivated early in embryogenesis, a process that normally occurs randomly (78). However, evidence suggests that skewed inactivation of the X chromosomes (>80% inactivation of one of the allele) occur in up to 43% of patients with KS (79). Skewed X chromosome inactivation may result in a silencing of either maternally or paternally imprinted genes, and it has been posited that some of the phenotypic variability in KS may be caused by this mechanism. However, the research to date does not support this hypothesis (30, 44, 72, 73, 80–82).

Polymorphism in the CAG repeat length in exon 1 of the androgen receptor gene has also been investigated for its relation to the phenotypic variability seen in KS, as the length of the CAG repeat is negatively correlated with the function of the androgen receptor (83). The existing literature shows that CAG repeat length of the androgen receptor does explain some of the variability seen in the phenotype of patients with KS, especially concerning anthropometry, with a positive correlation with height (80, 82), arm span (30, 80, 82), arm length (30), and leg length (30). Concerning other anthropometric measurements, however, such as bitesticular volume and gynecomastia (30, 82), as well as data regarding hemotology (30, 80, 82), lipid metabolism (30, 80), and bone-related parameters (80, 82), the findings are more inconsistent with some studies finding a negative

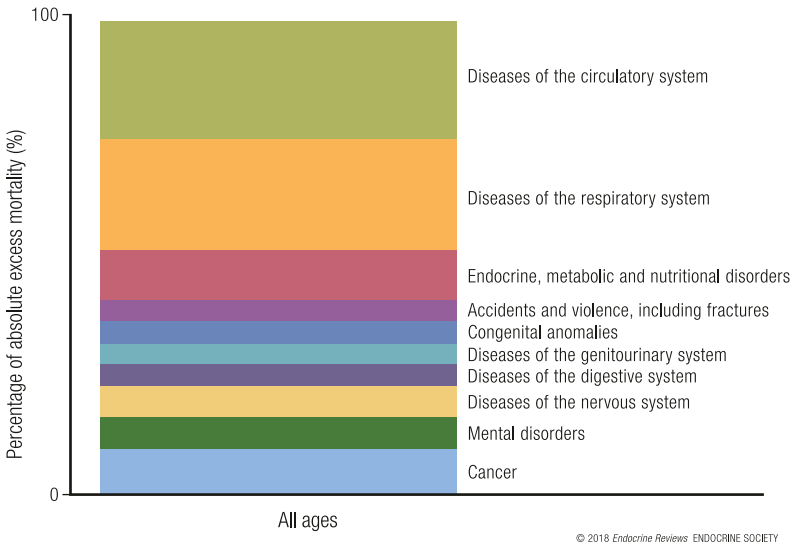


Figure 3. Differentiated excess mortality in KS for all age groups. Categories were defined according to ICD-9. Numbers are adapted to express the percentage of total absolute excess risk caused by the group of disorders in question. Includes data from Swerdlow *et al.* (4).

correlation between these measurements and CAG repeat length, whereas others found no correlation. Regarding cognitive function, no associations to CAG repeat length have been reported (72, 73). An association between CAG repeat length and response to testosterone therapy has also been found (82) but was not supported by another study (80). In addition to these findings, individual studies have also investigated and reported an inverse correlation between CAG repeat length and penile length (44), attained educational level, and chances of entering partnership (82). Wikström *et al.* (77) also found an inverse correlation between CAG repeat length and later onset of pubertal reactivation of the pituitary gonadal axis, which fits well with the finding of a positive correlation with height and arm span (30, 80, 82). Conclusively, CAG repeat length is related to different anthropometric measures and possibly also other measures, but remains a research tool. At present, it is not expected to become of manifest importance in the clinic.

Most males with KS have the karyotype 47,XXY (85% to 90%), whereas 6% to 7% have 46,XY/47,XXY mosaicism karyotype (1, 16) and the remaining 3% to 8% display either 46,XX/47,XXY or multiple X chromosome aneuploidy, including some with an additional Y chromosome (47,XXY/48,XXXY, 48,XXXY, 48,XXYY, 49,XXXXY). These latter cases display a more severe phenotype and should probably be considered outside the realm of KS diagnosis (1, 16) (Fig. 1). Boys and men with mosaicism have been described as presenting with a more favorable phenotype compared with nonmosaic KS males (29, 84); however, only one study has compared KS men with 47,XXY with KS men having 46,XY/47,XXY mosaicism (85). This study included only six KS men with mosaicism with the proportion of XY/XXY ranging from 2% to 87.5%. Here, KS men with 46,XY/47,XXY had larger testicular volume, lower levels of luteinizing hormone (LH) and estradiol, and higher mean total sperm count compared with nonmosaic KS males (azoospermic 93.0% vs. 96.3%), and none of them reported any comorbidity. Further studies are needed to characterize the phenotype of KS men with 46,XY/47,XXY. Regarding the 46,XX/47,XXY karyotype, the prevalence is very low, with only eight case reports published so far.

In addition to the previously mentioned genetic mechanism related to the X chromosome, CAG repeat length, and the karyotype, it has been suggested that the phenotype may be explained by X-linked escape genes, but the evidence is sparse. Thus far, only one gene on the sex chromosomes has been convincingly connected to the phenotype in KS, the *SHOX* gene, which explains some of the excess growth (86).

No genes or genetic mechanisms have been able to explain, for example, the increased risk of type 2 diabetes or attendant infertility and the cognitive and behavioral phenotype. Although the evidence for a gene dosage effect on the phenotype is largely missing, new support for this theory comes from Bellott *et al.* (68), who demonstrate that several genes on the Y chromosome have identical haplotypes on the X chromosomes. These genes could in theory be involved in the phenotype because they are expressed thrice in KS. Interestingly, Bellott *et al.* (68) found evidence that sex chromosomes may regulate gene expression throughout the genome due to enrichment of genes involved in transcription and translation, indicating that the phenotype seen in KS may be caused by a different expression of autosomal genes as well. Further evidence for this theory comes from a recent published study by Belling *et al.* (69), who evaluated gene expression in peripheral blood in men with KS and controls. They identified 363 differentially expressed genes in men with KS compared with controls, of which the majority was located on autosomal chromosomes. In addition, their analysis indicated dysregulation of genes involved in the immune system and energy balance, two areas associated with the phenotype in KS.

Although a gene dosage effect of having a supernumerary X chromosome may explain some of the phenotypic traits seen in KS, it cannot explain the variability seen in the clinical phenotype in KS, indicating that other mechanisms play a crucial role for the observed phenotype. Recently published data provided evidence that the DNA methylation profile in KS is associated with widespread changes both in blood and brain tissue (87, 88). It is possible that these genomewide alterations in DNA methylation play a role in the biological mechanism behind the clinical phenotype in KS, as well as its variability, as DNA methylation is part of our regulatory epigenetic machinery that is thought to affect our gene expression. More studies are needed to further elucidate these epigenetic perspectives on the phenotype, including studies of target tissues such as muscle, fat, brain, and testis, including both DNA methylation analysis and RNA expression analysis, as well as proteome analysis.

We recently presented data on epigenetics and RNA expression in blood from Turner syndrome individuals, which implicates several genes not hitherto thought to be involved in the phenotype of Turner syndrome (89), and these findings may have relevance for the conceptual thinking of genetics concerning KS. We found global hypomethylation of the genome, but also areas of hypermethylation and RNA expression changes. We speculate that the widespread hypomethylation of proximal promoters may have regulatory impact on gene transcription and suggest a possible link between the differential methylation and expression seen. In this study, the sex-chromosome analysis provided the largest existing set of differentially expressed genes, and in combination, these genes can be linked to several of the specific characteristics of Turner syndrome. They included

known escape genes such as *RPS4X*, *JPX*, and *LANCL3*. Other X chromosomal genes were differentially expressed (*CD40LG* and *KDM5C*). Because *KDM5C* participates in transcriptional repression of neuronal genes, we speculate that *KDM5C* may play a role in the distinct neurocognitive profile of Turner syndrome. In KS, we see something resembling a mirror image of the changes in Turner syndrome, with preferential hypermethylation, but affecting many of the genes also involved in Turner syndrome. We have also found pervasive RNA expression changes involving genes throughout the genome (unpublished material). Thus, a complicated picture of genotypic effects on phenotypes in KS is beginning to emerge, with epigenetic, RNA expression, and protein-protein changes. The precise impacts of these changes, and other genomic mechanisms, remain to be elucidated. A pictogram of the current understanding is presented in Fig. 4.

X chromosome inactivation and influence of the extra X chromosome

Among KS males, a global preferential hypermethylation of the genome seems to be present (67), although some CpG sites are hypomethylated, changes which can be thought of as orchestrated from the supernumerary X chromosome. Particularly for X chromosome inactivation, long noncoding RNAs are known to control the histone modifications that precede chromatin condensation and Barr body formation (90). Long noncoding RNAs regulate gene expression at many different levels, including through chromatin structure, transcriptional activation, and transcript stability (91). Noncoding RNAs often function via direct sequence complementarity with target transcripts and potentially target DNA regions. The overarching biological question related to KS is how to merge the understanding of the genome, epigenome, transcriptome, *etc.* with the different phenotypic manifestations related to different organs, viability, occurrence of congenital malformations, *etc.* Traits as diverse as type 2 diabetes, infertility, hypergonadotropic hypogonadism, congenital malformations, and altered neurocognitive performance remain unexplained.

Importantly, the research and thinking behind the genomics era have moved away from a focus on single genes on the supernumerary X chromosome for explaining the majority of phenotypes in KS to a focus on more subtle pervasive changes in the epigenome and transcriptome as the possible background (Fig. 4).

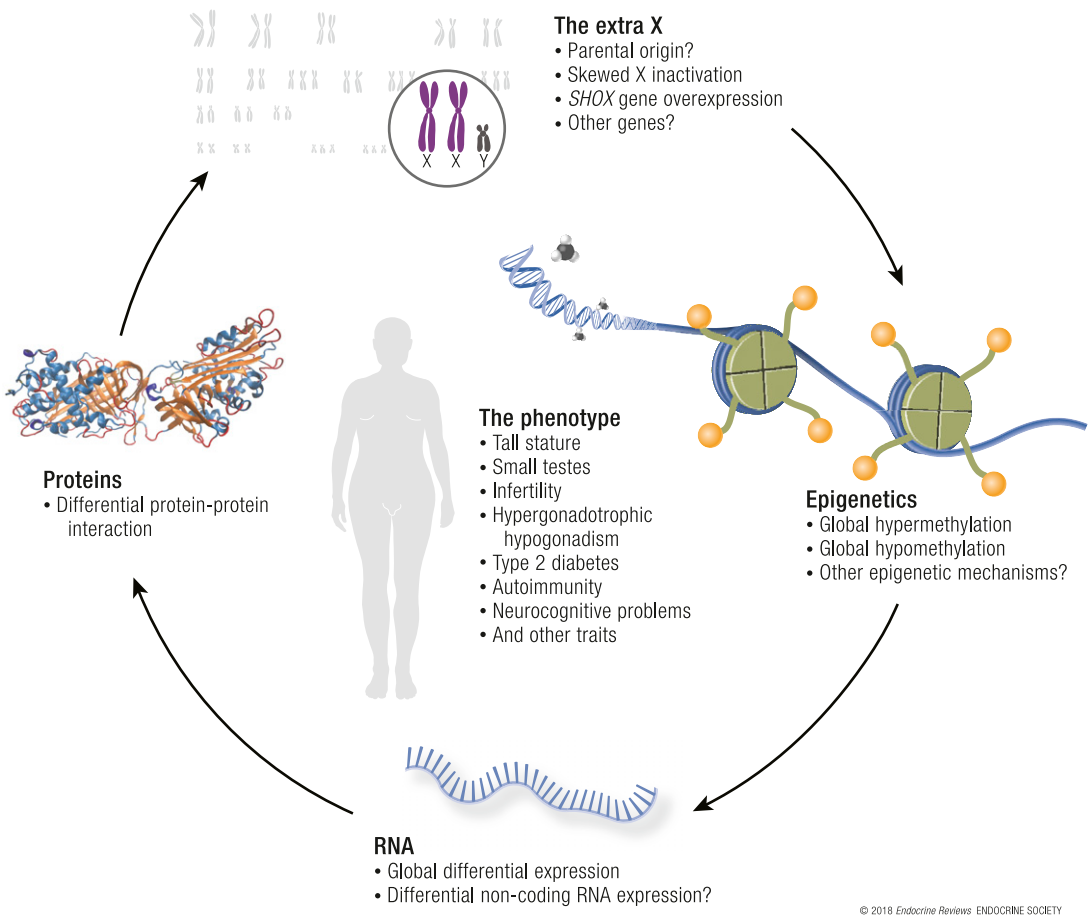


Figure 4. The figure depicts the current understanding of the genomics of KS, incorporating recent genomic results. Arrows depict possible, but not proven, pathways.

Summary of best evidence

The presence of three copies of the *SHOX* gene explains excess height in KS, and CAG repeat length is related to different anthropometric measures.

Areas of controversy

Genotype-phenotype relations in KS are largely unexplained. The current thinking that pervasive but discrete changes in the epigenome and the transcriptome explain parts of the phenotype still lacks compelling evidence. Yet other genetic mechanisms such as copy number variation or additional expression of escape genes on the extra X chromosome may be at play.

Hypogonadism and Metabolic Disease in KS

Although small testes are a hallmark of KS, many KS males pass through a normal or close to normal puberty without being detected. However, hypogonadism invariably presents problems sooner or later on in life.

Hypergonadotropic hypogonadism

Intrauterine and childhood hypogonadism

Whether hypogonadism is already present *in utero* is not clear. Studies point toward an altered 2D:4D (the length of the second-digit/fourth-digit ratio), suggesting a relative intrauterine hypogonadism (30, 92). Although controversial, the 2D:4D ratio has been suggested to be a surrogate marker of intrauterine androgen exposure (93). The anogenital distance may be a more precise measure of this, but at present there are no published data on anogenital distance in KS. Additional evidence with microphallus, cryptorchidism, degeneration of seminiferous tubules, and hyperplasia of Leydig cells suggests that even the KS fetus may often express lower levels of testosterone compared with male fetuses with normal karyotype (94), although measurements of amniotic fluid testosterone in a small group of KS fetuses, as well infants, were similar to controls (95, 96). A study of 4 to 12 year olds found that a large percentage of KS children had relatively low testosterone levels, and about 25% had relatively reduced penile length (97). In this same study, the authors found that elevated follicle-stimulating hormone (FSH) and low inhibin B were associated with a worse metabolic profile (97), indicating that emerging testicular failure and subsequent elevation of pituitary hormones could be linked to the metabolic syndrome, which is prevalent among adults with KS (see later) (32). At the onset of puberty, which occurs at the same time as in normal youth, the testes start to enlarge (46), soon to be followed by shrinkage despite elevated levels of FSH and LH (98), and testis size then remains much smaller than among normal adult males, with an average size of 2 to 4 mL (normative range: 15 to 30 mL) (29, 30).

Interestingly, a link between prenatal testosterone and adult facial features has been established, showing that a higher umbilical cord testosterone is associated with a more masculine facial structure and that the facial morphology was seemingly unaffected by adult testosterone levels (99). It would be very interesting to see if the intrauterine hormonal milieu is also revealed in the faces of KS boys and men.

Adulthood

Studies of adults with KS generally describe hypogonadism as being present in most males, but many KS males in fact have testosterone levels within normative ranges, and only when incorporating an elevated level of LH does a picture of compensated or relative hypogonadism emerge (100). The overwhelming majority of males with KS in outpatient clinics fulfill the criteria for hypogonadism (29, 30, 82).

Data from animal studies. In the two available mouse models of KS, testis weight is much smaller than in littermates (101, 102), whereas circulating testosterone was only significantly lower in aged KS mice (41,XX^{Y^*}) (102), seminiferous tubules were small and Sertoli cell only with absence of germ cells, and Leydig cell hyperplasia was present (101, 102), largely emulating human data.

Summary of best evidence. Data indicate that many KS males may be afflicted by relative hypogonadism much earlier in life than has previously believed and that hypogonadism is present long before the testosterone concentrations irreversibly level off around midpuberty.

Areas of controversy. There is need for large long-term follow-up studies of children with KS to determine the natural history of hypogonadism.

Weight and body composition

One obvious reason for the low rate of diagnosis in KS males is the lack of immediately recognizable physical features. Unlike the altered physical traits in autosomal trisomy, as in Down syndrome, only subtle changes in physiognomy are found in KS, although small, soft testes that differ in quality to normal testes are an unequivocally described trait in KS populations (Table 1). Typically, adult bitesticular volume is less than 10 mL with a mean around 4 to 7 mL (30, 103). However, in our experience, palpation of the scrotum is not routinely carried out in the male population, and even when small testes are in fact palpated, the possibility of KS is often not taken into consideration, which could be due to lack of knowledge about KS among physicians. Also, in preadolescent and adolescent KS, the difference in testicular volume between KS and normal males is less pronounced, although present (104).

Height in KS is increased with a mean of 5 to 7 cm compared with normal men (30, 105). However, the variability in height in KS is similar to normal men. The increased height is mainly based on an increased leg length (30), likely caused by delayed epiphyseal closing due to relative pubertal hypogonadism. The same mechanism also causes a relatively large arm span, sometimes exceeding height (28, 30). Increased height, however, has also been demonstrated already at ages 4 to 12 in KS boys (106), well before normal epiphyseal fusion, pointing toward an effect of other modulators, such as for instance *SHOX* gene dosage and increased number of CAG repeats in the androgen receptor (30, 80, 82).

In the original description of the syndrome, gynecomastia was found in all nine subjects and was deemed a characteristic trait (14). Later studies have found gynecomastia to be less common in KS, representing about a third of studied adult individuals (30, 31, 107, 108), although prominent or persisting pubertal gynecomastia remains an important sign of underlying KS. In a study of 25 boys with pubertal gynecomastia, three cases of KS were identified, all having prominent (Tanner $\geq$ B3) pubertal gynecomastia at a mean age of 13.8 years (109). Even earlier onset of gynecomastia in KS has also been observed (110). One recent study reported gynecomastia in 5 of 27 (18.5%) pubertal boys with KS, whereas none of 16 prepubertal non-KS boys presented with gynecomastia (104). In a Danish study, 16 out of 34 (47%) of KS adolescents (age <15.0 years) presented with gynecomastia (31), corresponding well with an American study finding gynecomastia in 8 of 18 (44%) KS boys under the age of 10 (74). Although the percentages vary between studies, it is clear that persistent gynecomastia during and even before pubertal transition is a clinical sign that should lead to suspicion of KS. It may also be that gynecomastia goes undetected, because many clinicians are relatively inexperienced in examining the breast area for gynecomastia.

Klinefelter *et al.* (14) originally found the nine index cases to be of both asthenic and normal build as well as obese, and in our experience, men with KS do in fact come in all sizes. Indeed, newer studies do collectively give evidence to KS males as generally having an increased fat mass compared with controls. In a cohort of 73 KS males, we found increased weight, hip, and waist circumference, increased total and abdominal fat mass, and increased total fat percentage compared with age-matched controls (30). Other studies have also found increased weight, waist circumference (32, 105), and total fat percentage (32) in KS males compared with controls. Studies of the KS phenotype typically find average body mass index (BMI) to be above the normal range in both adults (30) and boys (74), reflecting a tendency toward overweight. However, a recent Korean study found 57% of adult KS males to be within the normal BMI range (111). Average BMI in KS is likely to have been increasing over the years, as it has in the general population. Furthermore, it is worth noticing that BMI as an indicator of fat mass should be used carefully in KS, as the increased height and lower lean body mass hampers the usability of this parameter. This is demonstrated by KS males having an increased truncal fat percentage, evaluated by dual-energy x-ray absorptiometry, for any given BMI (Fig. 5) (30, 32). Testosterone treatment only partly compensates for this unbalance, because even after replacement therapy, total fat mass, abdominal fat mass, and total body fat percentage is still increased in KS males compared with controls with comparable BMI (30, 32, 112). Also, one study found no change in BMI or weight after 48 weeks of testosterone undecanoate in 19 men with KS (113). On the other hand, data has also been presented showing no significant difference in total body fat percentage between 48 testosterone-treated KS men and an equal number of healthy controls with comparable BMI (114).

The unfavorable skewness of fat mass and lean mass is present already in KS boys who also typically present with a BMI within the normal reference range (31, 112), but with underlying increased body fat and decreased lean body mass (112). Also, one study found higher frequency of BMI above the 85th percentile and waist circumference above the 90th percentile in pubertal compared with prepubertal KS boys (104). This finding, however, did not reach statistical significance, likely due to lack of power. Controversially, another study of 89 KS boys aged 7.5 $\pm$ 2.4 years did not find differences in body fat percentage or waist circumference compared with age-matched controls

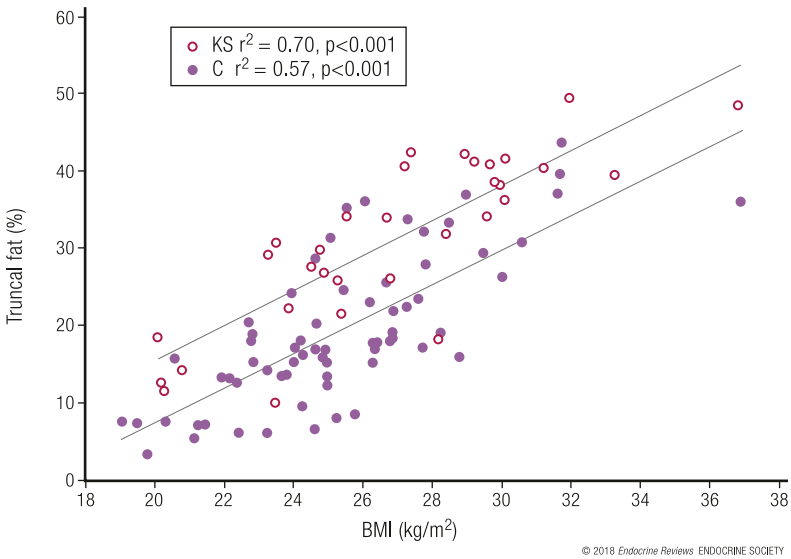


Figure 5. Truncal body fat in correlation with BMI. KS patients (red circles) have more truncal body fat (~8% more) for any given value of BMI than control subjects (C; purple circles). Reproduced with permission from Bojesen *et al.* (32).

(106). Irrespectively, a recently published randomized, double-blind, placebo-controlled study of boys with KS found lower body fat percentage in boys treated with oxandrolone (n = 46) vs. placebo (n = 47) (115). The boys in the placebo group were, on average, older and had higher body fat percentage at baseline, but the effects of treatment remained after statistically adjusting for these parameters. Both groups, however, reduced body fat percentage over the 2-year follow-up period.

Studies have reported other physical traits occurring more frequently in KS boys, including hypertelorism (69%), clinodactyly (74%), a high arched palate (37%), and elbow dysplasia (36%) (74, 104). As with gynecomastia, attendance to these subtle signs could perhaps improve the rate of an early KS diagnosis.

It is practically impossible to identify men with KS by simple visual observation, as the phenotype is often indistinguishable from men with normal karyotype, and for this reason, scrotal palpation should be more routinely carried out as a simple screening tool. Further, testosterone treatment introduced late in life seems to have little effect on body composition as a whole. The effect of testosterone treatment early in life on the adult phenotype has yet to be thoroughly examined.

Metabolic disturbances

Metabolism is classically seen as a balance between catabolism and anabolism. The relative hypogonadism in KS causes imbalance with a decreased anabolic potential hindering muscle buildup and metabolic turnover of sugar and fat (116). This hypoanabolic state impels a vicious cycle of abdominal fat deposition and global insulin resistance in the end causing increased morbidity and mortality (117), although short-term experimental hypogonadism does not induce insulin resistance (118), whereas a little longer-term (2 weeks) withdrawal of testosterone treatment in treated hypogonadal males does induce insulin resistance (119), and the issue of causality between hypogonadism and insulin resistance remains controversial. A central aspect of this vicious cycle is believed to be the development of insulin-resistant Leydig cells further compromising the already-hampered testosterone production in KS males (120). *In vitro* studies show a stimulatory effect of insulin on LH-induced testosterone production in both rat and mouse Leydig cells (121), and young insulin-resistant males produce less testosterone when stimulated with human choriogonadotropin (hCG) compared with nonobese men (122), indicating that insulin resistance also acts at the level of the Leydig cell.

However, the issue of what comes first, hypogonadism or obesity, has yet to be resolved. As described previously, it seems the propensity for obesity in KS is present early on, before hypogonadism is properly stratifiable. Thus, researchers have some way to go before the natural history of this viscous cycle in KS is properly understood.

Motor control, muscle, and strength

KS males often suffer from nonspecific motor impairments such as reduced muscle strength, running speed, agility, and coordination (72, 103) and a large prevalence of essential tremor (40). The underlying cause for these changes is unknown.

Reduced muscle buildup has been demonstrated by the finding of decreased lean body mass and intermuscular adipose tissue–free skeletal muscle mass in untreated KS males compared with controls (32, 33). In the same study, muscle strength (right biceps and right quadriceps) was reduced to approximately 80% and maximum oxygen consumption to 70% in 70 men with KS compared with 70 age-matched controls. Likewise, exercise capacity, expressed by workload capability, and oxygen consumption were significantly impaired when comparing 48 testosterone-treated KS males with age- and BMI-matched controls (114).

The lack of muscle buildup seems to be evident already during childhood, as one study found mild hypotonia in 62% and severe hypotonia in 15% of KS boys (74). A decreased muscle mass at the lower leg was especially noted. The findings, however, were not correlated to testosterone, perhaps lending to the theory of impaired cerebral motor function as an etiological factor for decreased muscle tone in KS boys (72, 124). Also, in a very recent randomized, placebo-controlled study, no effect was seen on strength after 2 years of low-dose oxandrolone treatment in 43 KS boys ages 6.9 $\pm$ 2.2 years (125).

No study has to our knowledge looked at muscle buildup in KS men vs. controls following a standardized training regimen, although we would expect it to be significantly impaired in KS males. In addition, it would be interesting to investigate the potential for testosterone treatment to better exercise capacity in KS males.

Glucose and insulin

Plasma fasting glucose has been found to be increased in adult KS males, with more KS males than controls having fasting glucose in the prediabetic or diabetic range (32, 126). In boys with KS (4 to 13 years), however, fasting glucose appears not to be impaired (106). The changes in glucose levels are followed by higher serum insulin and homeostatic model assessment-insulin resistance (HOMA-IR), a measure of insulin resistance, in KS males compared with controls (32, 105, 114, 126–128). Hyperinsulinemia and insulin resistance in KS have also been demonstrated using a hyperinsulinemic euglycemic clamp (129). In this study of five men with KS, testosterone level was found to independently predict insulin-dependent glucose disposal

(129). Testosterone treatment seems to only slightly improve fasting glucose values in KS (32, 126, 130).

In one study, HOMA-IR was insignificantly higher among testosterone-treated KS males compared with untreated KS males (32). In another study, both mean serum insulin and HOMA-IR were numerically higher in treated vs. untreated KS males (no statistics applied) (114), and in yet another study, KS males on testosterone treatment had lower HOMA-IR than the untreated group (105), indicating some effect of treatment. Similarly, HOMA-IR, but not serum insulin, was reduced in a group of 56 men with KS after 18 months of testosterone treatment (128). Thus, the data on the effect of testosterone treatment on features of carbohydrate metabolism in KS are somewhat conflicting. Also, although some studies have demonstrated an effect of testosterone treatment on insulin sensitivity in healthy and in obese men, it is likely that the effects in KS are indirect via a long-term reduction of visceral fat mass (131) (see previous "Weight and body composition" section).

There are indications of a considerable genetic component, as one study found that increased insulin resistance (higher HOMA-IR) in KS males was related to gene dosage of the *CSF2RA* gene located on both the X and Y chromosomes. Interestingly, higher HOMA-IR was seen when the supernumerary X chromosome was of paternal vs. maternal origin (105). Further, a study in KS boys demonstrated insulin resistance (HOMA-IR $\geq$ 2.5) in 24% of KS boys down to an age of 5 years (106). This lends to a significant genetic contribution rather than impairments of carbohydrate metabolism, solely due to a vicious cycle of hypogonadism, induced fat deposition, and sarcopenia. However, in the same study, HOMA-IR was found to be increasing with age as the relative hypogonadism becomes more prominent (106).

Higher leptin has been demonstrated in KS males compared with controls, with no effect on leptin levels after 3 months of treatment with intramuscular injections of an androgen compound (132). However, cross-sectional data show a tendency toward lower leptin after testosterone treatment when comparing untreated KS with long-term, testosterone-treated KS (32), without normalization of leptin levels even after long-term testosterone. In addition, ghrelin was seen to be normalized in seven hypogonadal men, whereof four had KS after 6 months of testosterone treatment (133). A possible mechanism for insulin resistance in KS has been proposed in a study finding overproduction of CCL2, a small chemokine expressed at sites of inflammation and associated with insulin resistance, in KS males compared with controls (134).

Conclusively, larger long-term, prospective, randomized, controlled studies are needed to clarify the effects of testosterone treatment on glucose metabolism and insulin resistance in KS, taking into account genetic aspects, changes in body composition, and measurement of associated hormones, including appetite-regulating hormones such as leptin.

Metabolic syndrome

Although data on individual metabolic parameters in KS might be mixed, there is no doubt that, ultimately, men with KS are highly susceptible for developing metabolic disorders, namely the metabolic syndrome and type 2 diabetes. Increased prevalence of the metabolic syndrome has been demonstrated in numerous studies. In a study by Bojesen *et al.* (32), 44% of KS males were classified as having metabolic syndrome according to the criteria given by the National Cholesterol Education Program/Adult Treatment Panel III, reflecting a fivefold increased risk. This finding was corroborated in later studies using the same criteria (114, 135), the International Diabetes Federation 2004 criteria (130), and the 2009 harmonized criteria (105). Especially truncal obesity seems to predict development of the metabolic syndrome in KS males (32). In KS boys, one study found the metabolic syndrome to affect 8%, with further as much as 36% only missing to fulfill one criteria for the diagnosis. The youngest boy who met the criteria was 4 years old (106).

No study has aimed specifically at evaluating the effects of testosterone treatment on preventing development of the metabolic syndrome. Still, available data are not promising. Three studies have found statistically nonsignificant but numerically higher rates of metabolic syndrome in treated vs. untreated men with KS (105, 114, 130). For instance, one study found the prevalence of the metabolic syndrome to go up from 30.8% to 38.5% after a median duration of 4 years of testosterone treatment (130). It is of course impossible to say if the increase would have been even greater if testosterone treatment had not been initiated, and the vicious metabolic cycle had perhaps prevailed. Longitudinal studies are needed to clarify these associations.

"The effect of testosterone treatment early in life on the adult phenotype has yet to be thoroughly examined."

It is as well still unclear which etiological factors are most important in causing the metabolic syndrome associated with KS. One study has demonstrated higher prevalence of the metabolic syndrome with higher expression of the differentially expressed gene *CD99* associated with sex-dependent induction of inflammatory conditions (105). Interestingly, in those KS males with the highest expression of *CD99*, testosterone treatment in itself was a predictor of an increased likelihood for the presence of the metabolic syndrome. However, actual blood testosterone levels were not associated with the prevalence of the metabolic syndrome (105). Furthermore, in the study by Bojesen *et al.*, the association between hypogonadism and features of the metabolic syndrome disappeared after controlling for truncal fat percentage (32). On the other hand, another study found measures of the metabolic syndrome in KS to be correlated with levels of insulin-like factor 3, a small peptide hormone

secreted only in Leydig cells and as such a biomarker of Leydig cell function and thus indirectly testosterone production (136). Conclusively, the incidence of the metabolic syndrome in KS seems higher than in other populations of hypogonadal men (137), further lending toward a syndrome-specific (genetic) background.

Diabetes

Consistent with other findings regarding metabolic syndrome, the prevalence of type 2 diabetes is found to be increased in KS, and studies reporting on increased prevalence of the metabolic syndrome also find higher prevalence of type 2 diabetes (32, 135). Epidemiological studies of both morbidity and mortality have found occurrence of diabetes in KS to be more than threefold increased (4, 9). A clinical study of 39 men with KS found the prevalence of diabetes to be 12.5% (130), with an early average age at diagnosis of 27.1 years, and a higher prevalence than in a control group of men with idiopathic hypogonadotropic hypogonadism. Testosterone treatment did not seem to better glucose levels. The authors thus speculate that testosterone deficiency alone cannot explain the marked increase of type 2 diabetes in KS, and it even lends to a possible effect of X chromosome dosage, as even higher prevalence of diabetes has been recorded among patients with more X chromosomes (130).

Interestingly, occurrence of type 1 diabetes also seems to be increased (9). This latter finding is supported by the recent finding of autoimmune antibodies directed against diabetes-specific autoantigens in 8.2% men with KS compared with less than 1% of controls (37). Apart from this, very little is known about the development, treatment, and prognosis of type 1 diabetes in KS.

Although the epidemiological data indicate that type 2 diabetes and the attendant comorbidity, for instance, could be central to the increased mortality seen in KS, no studies have been conducted to evaluate the course of the disease or the efficacy of antidiabetic treatments in KS. One obvious reason for this is the need for large patient cohorts to be able to include enough men with KS and concomitant diabetes.

Lipids

Men with KS often have dyslipidemia. In a recent German study of 132 men with KS, an unfavorable lipid profile was described with increased triglycerides and decreased high-density lipoprotein (HDL) cholesterol compared with both male and female controls (105). Similar results are seen in the Danish cohort (32), but it could not be confirmed in an Italian study of 121 men with KS (128). However, support for an unfavorable lipid profile as an intrinsic part of KS comes also from studies in boys with KS. One study found 37% of prepubertal boys with KS with elevated low-density lipoprotein (LDL) and 65% with low HDL (106). These findings have recently been confirmed in another study from the same group, additionally finding elevated triglycerides in 16% of 93 boys with KS ages 4 to 12.9 years (115). Dyslipidemia was seen in 18% of KS boys with concomitantly elevated triglycerides and low HDL in another study (97).

The only study designed to clarify the effects of testosterone treatment on lipid fractions in KS looked at treatment effects after 1 and 4 weeks, but only included 10 men with KS (138), and showed an increase in total cholesterol after 4 weeks, whereas other fractions of cholesterol and triglycerides remained unaltered. Even in men with normal karyotype, the effects of testosterone treatment on lipid fractions are not clear, although some formulations, like intramuscular injections, have been associated with a reduction in HDL (139, 140). Comparisons of lipid fraction between untreated and treated KS adults and controls are listed for studies covering a total of 342 untreated KS males and 454 treated KS males (Table 2). It can here be appreciated that substantial heterogeneity in lipid profile exists between cohorts of men with KS. However, it also seems that testosterone treatment in KS could be increasing triglyceride levels. A single study found triglycerides to decrease after testosterone treatment, but this might be caused by selection bias, as the population as a whole at baseline had a mean triglyceride level at 117.6 ± 169.9 mg/dL, whereas the subset of patients followed before and after treatment at baseline had a mean triglyceride level of 311.5 ± 500.5 mg/dL. It is thus significantly higher than the population as a whole and the normal reference range, <150 mg/dL, provided by the authors (128).

Testosterone treatment in KS seems to cause a decrease in HDL in observational studies (Table 2), which precludes conclusions concerning causality. It might be that alterations are only seen after long-term treatment and thus not observed in studies with only few years follow-up. However, a recent randomized, double-blind, placebo-controlled trial in prepubertal KS boys found lower HDL cholesterol after 2 years in the treated vs. the placebo group ($P < 0.001$) (115).

Interestingly, lipid fractions in KS do not seem to be directly correlated to testosterone levels (135) nor to certain genetic aspects including paternal origin of the supernumerary X chromosome, skewed X chromosome inactivation, or number of androgen receptor CAG repeats (30). Hence the mechanism causing the dyslipidemia in KS is still largely unknown.

Taken together, KS males present with an unfavorable lipid profile, similar to what is seen in type 2 diabetes, with high total cholesterol especially due to an elevated LDL fraction with a decreased HDL fraction and also increased triglycerides. Most of the evidence concerning the effect of testosterone treatment is from observational studies and thus of low quality. There is a definite need for large long-term,

Table 2. Lipid Profile in Studies of KS and the Effect of Testosterone Treatment

			Triglycerides			Total Cholesterol			LDL Cholesterol			HDL Cholesterol		
Reference	Study Type	n	U vs. T	U vs. C	T vs. C	U vs. T	U vs. C	T vs. C	U vs. T	U vs. C	T vs. C	U vs. T	U vs. C	T vs. C
Yesilova *et al.* (141)	Longitudinal, prospective	32	NS	NS	—	0.011 (U↑)	NS	—	—	—	—	—	—	—
Bojesen *et al.* (32)	Cross-sectional	U: 35 T: 35	NS	0.0001 (U↑)	—	NS	0.002 (U↑)	—	0.04 (U↑)	0.0001 (U↑)	—	NS	0.0001 (U↓)	—
Aksglaede *et al.* (31)	Cross-sectional	U: 15 T: 56	NS	—	—	NS	—	—	NS	—	—	0.037 (U↑)	—	—
Pasquali *et al.* (114)	Cross-sectional	U: 21 T: 48	—	—	NS	—	—	NS	—	—	NS	—	—	NS
Jiang-Feng *et al.* (130)	Longitudinal, retrospective	39	NS	—	—	NS	—	—	NS	—	—	NS	—	—
Selice *et al.* (128)	Longitudinal, prospective	121[a]	<0.05 (U↑)	NS	—	NS	NS	—	—	—	—	NS	NS	—
Jørgensen *et al.* (142)	Cross-sectional	U: 21 T: 41	0.04 (U↓)	NS	—	NS	NS	—	—	—	—	0.01 (U↑)	NS	—
Chang *et al.* (30)	Cross-sectional	U: 23 T: 50	0.007 (U↓)	—	0.003 (T↑)	NS	—	NS	—	—	—	0.002 (U↑)	—	0.001 (T↓)
Zitzmann *et al.* (105)	Cross-sectional	U: 35 T: 97	NS	—	—	—	—	—	NS	—	—	<0.05 (U↑)	—	—

Abbreviations: C, controls; NS, not significant; T, treated KS; T↑, highest value in treated KS; T↓, lowest value in treated KS; U, untreated KS; U↑, highest value in untreated KS; U↓, lowest value in untreated KS.
[a]Fifty-six KS males were treated and followed.

randomized studies to clarify the effect of testosterone on lipids and other metabolic parameters in KS.

Data from animal studies. There are no data from animal studies to support the specific metabolic profile of humans with KS. Mouse models would be valuable in further studying the metabolic changes seen especially in adult KS males.

Summary of best evidence. The risk of type 2 diabetes is elevated four- to sixfold, the metabolic syndrome, including overweight and frank obesity, is frequently seen, and the lipid profile of many KS males is unfavorable.

Areas of controversy. It is not clear whether the unhealthy metabolic profile is intrinsic to KS and dependent on the underlying genetics or in part due to shorter or longer periods with hypogonadism or is partially explained by unfavorable socioeconomic conditions. We believe that unraveling the genomics of KS will lead to new insight and possibly identify unique pathways for the development of type 2 diabetes.

Cardiovascular disease

A substantial part of the increased mortality seen in KS is based on higher prevalence of cardio- and cerebrovascular disease (CVD). This has been demonstrated by epidemiological studies on morbidity and mortality (Fig. 2) (4, 9). CVD continuously accounts for approximately 30% of deaths in the United States, and the epidemiological studies have found the overall risk for CVD in KS males to be increased approximately twofold (4, 8). Thus, proper management and prevention of these diseases seem pivotal. Recently, one model for cardiovascular assessment in KS has been proposed (13), and such initiatives are needed in an effort to secure the health of men with KS.

The heart

The basis for cardiovascular health must be a healthy heart. In Turner syndrome (45,X), the most common female sex chromosome anomaly, congenital cardiac anomalies are central to the pathology (143). Several cases of cardiac anomalies have also been reported in KS (144–148). Swerdlow *et al.* (4) found mortality due to congenital cardiovascular anomalies to be more frequent in KS males [standardized mortality rate (SMR): 7.3; 95% CI: 2.4 to 17.1] compared with expected rates in the British population. In addition, malformations of the heart were found to be more frequent in KS males in a study by Bojesen *et al.* (9). We speculate, however, that the higher rates of congenital malformations found in KS males may be due to selection bias, as newborns exhibiting heart deficits are more likely to undergo chromosomal examination. Along the same lines, two studies by Fricke *et al.* (35, 149) found mitral valve prolapse to be more frequent

in KS males. These findings, however, have not been replicated in more recent studies with a larger sample (36, 114). Impairment of ventricular diastolic function and chronotropic incompetence in KS, on the other hand, have been reported in two large echocardiographic studies (36, 114). The association between these findings and features of androgenicity, including testosterone treatment and the metabolic syndrome, remains to be elucidated. Pasquali *et al.* (114) consistently described chronotropic incompetence in KS males irrespective of treatment status, whereas Andersen *et al.* (36) found a reduction in androgenicity to be as well associated with reduction in heart function and further accentuated in KS individuals with the metabolic syndrome.

Shortening of the QTc interval (time between the start of the Q wave and the end of the T wave in the electrical cycle of the heart, corrected for heart rate) in KS males compared with both male and female controls has also been reported by two independent recent studies (87, 137). Short QTc interval is associated with an increased risk of cardiac arrhythmias and cardiac arrest. In the study by Jørgensen *et al.* (142), a marked effect of testosterone treatment was seen in treated men with KS having shorter QTc than in untreated men with KS, reflecting the notion of the inverse correlation between QTc and endogenous testosterone found in other populations (150). However, in the study by Zitzmann *et al.* (105), no difference in QTc interval was observed between treated and untreated men with KS, and QTc was not associated with testosterone levels. In this study, however, shorter QTc was seen in those with paternal origin of the supernumerary X chromosome and those expressing higher levels of genes differentially expressed between KS males and controls.

As such, it is not clear to what extent the KS karyotype in itself is affecting heart function and to what extent the observed effects are due to metabolic changes and hypoandrogenism. In addition, further prospective, preferentially randomized, studies are needed to clarify the effect of testosterone treatment on heart function in KS.

The vasculature

Testosterone is a highly vasoactive hormone, functioning primarily as a vasodilator, with an apparent protective effect against atherogenesis (151). Accordingly, Foresta *et al.* (152) demonstrated reduced luminal diameter in the brachial artery, common carotid artery, common femoral artery, and abdominal aorta in 92 untreated KS males compared with controls. Still, matters were complicated by the fact that no difference was seen among KS males when stratifying in two groups with normal or subnormal testosterone levels, respectively (152). The authors thus speculate that X chromosome gene dosage could be involved in the pathology, because the opposite dilation of major arteries is seen in 45,X Turner syndrome (143). Whether testosterone treatment is capable of increasing arterial diameter in KS males has not been investigated.

Moreover, one study has found a reduced number of endothelial progenitor cells in KS males compared with controls (153). These cells play a role in repairing and regenerating the endothelial vessel lining, and a reduction in cell count could thus reflect impairment of the endothelial vessel wall. The antiatherogenetic effect of testosterone is believed to be exerted through anti-inflammatory mechanisms. This is supported by studies in males finding an inverse relationship between serum testosterone levels and levels of proinflammatory cytokines in patients with coronary artery disease, type 2 diabetes, and/or hypogonadism (151). The causal relationship between testosterone and atherosclerosis is, however, still unclear. Recent data from a randomized and placebo controlled multicenter study (the Testosterone Trials) was published, finding testosterone treatment in elderly hypogonadal men to be associated with a greater increase in coronary artery noncalcified plaque volume (154), to some extent obscuring the image of testosterone as being protective against atherosclerosis. Presence of atherosclerosis has not been systematically studied in KS males. However, in the study by Foresta *et al.* (152), no difference was seen in carotid intima-media thickness (cIMT) between untreated KS men and controls. Further, no difference was seen when stratifying for testosterone level. cIMT is a marker of atherosclerotic disease, and interestingly, another study from the same group published the same year found cIMT to be increased in 48 testosterone-treated KS males compared with controls (114). It is noted, however, that the controls in the latter study had much lower cIMT values than those in the first study and that cIMT in the latter study further did not differ between treated and untreated KS males (114, 152).

Based on the morbidity pattern seen in KS, with especially a high incidence of metabolic disorders, it would seem likely that atherosclerosis and risk factors hereof, including hypertension, should also be frequent in KS males. Still, as perhaps partially demonstrated by the lack of clear evidence for susceptibility to atherosclerosis, blood pressure is seemingly not increased in KS males when compared with controls. Mean blood pressure is typically within the normal range (135, 155) and does not differ significantly from that of matched control populations (32, 114, 128, 152, 153, 156). In addition, in boys with KS, hypertension is not found in spite of other features of the metabolic syndrome being more or less frequent (106, 115). In a Korean study, however, hypertension was reported in 15.2% of 376 men with KS, and in the group with BMI above 25, hypertension was found in 18.8%. This is somewhat surprising as the worldwide prevalence of hypertension according to the World Health Organization (WHO) is approximately 40%. Previous

epidemiological studies have not looked at prevalence of hypertension, but it would be very interesting to get a more precise estimate of this from a large cohort of men with KS on and off testosterone treatment.

The finding of normal levels of adiponectin (32, 114) is in further support of normal blood pressure in KS males. Low levels of adiponectin are associated with hypertension and metabolic disorders. In addition, adiponectin, produced in fat cells exclusively, is negatively associated with testosterone and as such suppressed by testosterone treatment (120). Subsequently, one study found comparable but slightly increased adiponectin levels in untreated KS males vs. slightly suppressed levels in treated KS males (126). It thus seems that the relative hypogonadism in KS could perhaps be protective against hypertension through outbalancing the reduction in adiponectin levels seen with metabolic disorders.

Venous thrombosis

Not surprisingly, the incidence of venous thrombosis (VTE) is increased in KS. An increased frequency of venous leg ulcers, DVT, and pulmonary embolism (PE) in KS was first described by Campbell *et al.* (157) and Campbell and Price (158). Later, epidemiological studies of mortality and morbidity confirmed that the risk for VTE in KS is raised four- to eightfold (4, 9). Recently, this was corroborated by a Swedish epidemiological study finding an adjusted standardized incidence ratio of 6.43 (5.15 to 7.93) for VTE in a cohort of 1085 men with KS comparing the observed events with the expected number of events based on the national prevalence among Swedish males (7). Interestingly, the authors found an especially high relative risk for VTE in age groups <30 and 30 to 49 years of age [standardized incidence ratio 12.10 (6.22 to 21.21) and 11.00 (7.86 to 14.99), respectively]. Further, the cumulative incidence of VTE in KS at age 70 was 20.8%.

The 30-day and 1-year mortality rates after VTE are around 10% and 20%, respectively (159), and the collective evidence indicates that VTE in KS is significantly increasing the risk of death already at a young age. Furthermore, the Swedish study did not find any difference in VTE incidence before or after diagnosis of KS (7). This is an important finding opposing the simple explanation that the increased incidence of VTE in KS should be due to elevated hematocrit as a consequence of poorly managed testosterone treatment (160). Taking into account the KS phenotype with its specific morbidity pattern and hormonal profile, it seems fair to assume that a distortion of the hemostatic balance should be in effect. The prevailing hypothesis evolves around obesity and metabolic challenges in KS, leading to decreased fibrinolytic capacity caused by increased levels of plasminogen activator inhibitor-1 (PAI-1) (161). High BMI and low testosterone levels are associated with increased levels of PAI-1 (162, 163), and two studies have indeed found higher levels of PAI-1 in KS males with leg ulcerations compared with KS males without leg ulcerations (164), and in KS males compared with both male and female controls (105). Yet, it is still unclear whether these observations are simply associated with fat mass or some intrinsic effects of the KS karyotype. Interestingly, in the study by Zitzmann *et al.* (105), the levels of PAI-1 in testosterone-treated and untreated KS males were comparable, indicating that testosterone treatment perhaps is not capable of restoring fibrinolytic capacity. It is also possible that an effect of the altered hormonal composition in KS with a relatively elevated estrogen could potentially alter the hemostatic balance. In this context, it is also interesting to consider aromatization of exogenous testosterone compounds. Further, higher levels Factor VIII or Factor IX could be hypothesized, because the genes for these coagulation factors sit on the X chromosome. Although increased levels of Factor VIII were described in a recent case series of VTE in six men with KS (165), the authors conclude that the frequency of thrombophilia markers in the six KS cases did not differ from a control population with VTE. Thus, larger studies evaluating the hemostatic balance in KS are needed. To this end, we are currently conducting one such study aiming at evaluating aspects of coagulation and fibrinolysis in a larger cohort of men with KS, before and after treatment with testosterone (ClinicalTrials.gov no. NCT02526628).

In clinical practice, we consult with specialists in hemostasis and coagulation and normally continue testosterone therapy among men with KS and VTE, with appropriate addition of anticoagulation therapy, and as such, the clinical handling does not differ from clinical guidelines within the area.

"Low levels of adiponectin are associated with hypertension and metabolic disorders."

Arterial thrombosis

Diabetes, obesity, unfavorable lipid profiles, and an increased prevalence of autoimmune conditions are all characteristics of KS, as well as risk factors for cerebral stroke and myocardial infarction. The evidence for an increased incidence of arterial thrombosis in KS is, however, less convincing than what is the case for VTE. This is, on the other hand, in line with the lack of convincing evidence for other arterial thrombosis risk factors, atherosclerosis and hypertension, being more prevalent in KS.

Price *et al.* (166) found mortality from diseases of the circulatory system to be increased in KS males. This was mainly attributed to an increased mortality from cerebrovascular disease, whereas the incidence of ischemic heart disease was insignificantly increased. Swerdlow *et al.* (4) found mortality from cerebrovascular disease to be increased in KS males [SMR (95% CI) 2.2 (1.6 to 3.0)], whereas mortality from cardiovascular disease was slightly decreased [SMR (95% CI) 0.7 (0.5 to 0.9)]. Bojesen *et al.* (9), on the other hand, found a higher incidence of ischemic heart

disease in KS males [HR (95% CI) 1.71 (1.28 to 2.29)], but only an insignificant increase in incidence of cerebrovascular disease [HR (95% CI) 1.19 (0.78 to 1.81)].

Men with KS present with a high incidence of several factors increasing the risk for atrial fibrillation, which would support a higher incidence of stroke. However, no studies have properly investigated the incidence of atrial fibrillation in KS.

In addition, when considering the incidence of arterial thrombosis in KS, it is important to consider measures of primary prevention. It is likely that men with KS are, in fact, as a consequence of the morbidity pattern, more frequently offered preventive measures against arterial thrombosis (*e.g.*, acetylsalicylic acid or statins).

Furthermore, Di Minno *et al.* (156) recently demonstrated increased platelet aggregation in testosterone-treated men with KS compared with controls. We, on the other hand, have, in an ongoing study, found no evidence for increased platelet aggregation in a group of untreated men with KS (167). Further, treatment with supraphysiological doses of testosterone has been found to increase total homocysteine levels, which are also associated with an increased cardiovascular risk, in a group of 32 men with KS (141). Thus, in regard to the ongoing debate about safety of testosterone treatment, more knowledge is needed on how testosterone treatment affects cardiovascular disease risk in KS.

Data from animal studies. As for metabolic diseases, there is no evidence from animal studies, but also in this case, it can be expected that such data would be of value to enhance our understanding of heart and vessels in KS.

Summary of best evidence. The risk of DVT and PE is clearly elevated in KS.

Areas of controversy. Whether the risk of heart and cerebrovascular disease is clearly elevated in KS is not clear, and further large epidemiological studies would likely elucidate this area. There is also a need for further studies of the coagulation system in KS and the effects hereon of TRT.

Bone metabolism

Because KS males all eventually develop relative or manifest hypogonadism, and relatively low testosterone and estradiol levels, it is to be expected that bone metabolism will be affected. Not surprisingly, fractures and osteoporosis occur more frequent among KS males (4, 8, 9). Many clinical studies have looked at bone mineral density (BMD) as a proxy and generally found this to be decreased (33, 168), although no clear relation between serum testosterone and BMD has been found. We also found both serum markers of bone formation and bone resorption comparable to controls (33). We and others have also described decreased 25-hydroxy-vitamin D and muscle strength (33, 169), and in addition to this, we have demonstrated low insulin-like factor 3, a new marker of Leydig cell function, in both treated and untreated KS, which was correlated with osteocalcin, a marker of bone formation, although no direct correlation was seen between insulin-like factor 3 and BMD (136).

Using high-resolution peripheral quantitated computed tomography (pQCT), we recently demonstrated distinct differences between KS males and controls and showed that KS males had low volumetric BMD and especially reduced trabecular density at the tibia. Furthermore, we described the findings as being similar to what is seen in postmenopausal women, where a compromised trabecular network with low trabecular number is seen. The findings resulted in lower bone strength at the level of the tibia (170). To date, there are no randomized clinical trials investigating the effect of appropriate bone-healthy treatment with testosterone, 25-hydroxy-vitamin D, and calcium supplementation among KS males, but given the available data from other conditions and from observational studies (34, 169, 171), it seems prudent to assume that such treatments will help in keeping fractures and osteoporosis at bay.

Data from animal studies

The adult 41,XXY mouse shows changes in bone morphometry with reductions in bone volume and thinner trabeculae, which resemble changes in human KS (172).

Summary of best evidence

Osteoporosis is more frequent in KS and the likely result of hypogonadism and thus relatively low levels of estradiol.

Areas of controversy

It remains to be seen if appropriate TRT will normalize the microarchitecturally unfavorable changes of the bone observed in KS.

Autoimmunity

The prevalence of autoimmune disease is also increased in KS in the presence of surplus X chromosome material (38), just as it is in Turner syndrome, with a lack of X chromosomal material (173). This increase in autoimmune disease is likely due to extra X chromosomal material in KS, although no genes or genetic mechanisms have been identified. Similarly, in Turner syndrome, lack of X chromosomal material is thought to be the basis for the increased predilection for autoimmune disease (173). In a large UK registry study, Addison disease, type 1 diabetes, multiple sclerosis, hypothyroidism, rheumatoid arthritis, Sjøgren syndrome, and systemic lupus erythematosus were all identified more frequently among KS males compared with the male background population (38), with similar findings in a Danish registry study (9). Others have also noted a much higher frequency of

systemic lupus erythematosus (39, 174), and a recent clinical study found a higher frequency of antibodies related to type 1 diabetes (37). Future studies should aim at dissecting the pathogenesis behind autoimmune disease and KS.

Fertility and Sexual Function

Fertility

Men with KS usually have small testicles and azoospermia (29) and account for about 10% of azoospermia cases (175). In literature, a few cases have been described where pregnancy was achieved during natural conception (176) or after intracytoplasmic sperm injection (ICSI) using ejaculated sperm (29, 177). The couple described by Crüger *et al.* (177) actually had another child by ICSI a couple of years later. At each treatment, about six sperm cells were found in the ejaculate. KS men with sperm in the ejaculates may be suspected to have chromosomal mosaicism in the testicles or in general. In this first case describing paternity by natural conception in KS, there is no mentioning of how many peripheral leukocytes the diagnosis was based on. Furthermore, the man with KS reported in this study had testicular volumes of 10 mL, which is unusually large for a KS man (176). Therefore, it cannot be excluded that this man might have a KS mosaicism within the blood and/or inside the testis. In other studies, however, testicular sperm has indeed been detected in KS men who probably did not have mosaicism in peripheral blood (177, 178).

To date, it is not clear what actually happens in the KS testis: Do 24,XY spermatogonia have the ability to complete meiosis, or do the normal spermatozoa found in some adults with KS arise from patches of normal 23,X/23,Y spermatogonia? This latter possibility would suggest that cryptic mosaicism is present in KS, a hypothesis that so far has not been supported by much data (179). In one study of KS males with ongoing spermatogenesis, it was found that viable germ cells were euploid, but were surrounded by apparently normal functioning 47,XXY Sertoli cells (180), and the overwhelming majority of tubuli were devoid of germ cells. Similarly, it has been demonstrated in a preliminary report that intratesticular testosterone levels among KS males are higher than among controls, with a fourfold higher number of Leydig cells per tubule and a similar number of Sertoli cells per tubule, and with a tubule thickness that was twice the size of controls (181). A recent study in mice showed that trisomy-biased chromosome loss occurs quite frequently in induced pluripotent stem cells in XXY and XYY mice and that these euploid XY pluripotent stem cells can develop into male germ cell lineage and become viable sperm, leading to seemingly normal offspring (182). The authors also demonstrated that this trisomy-biased chromosome loss can occur in human KS fibroblasts, and thus a similar mechanism could be present in KS males, explaining the presence of ongoing normal spermatogenesis (182).

It is not understood how the extra X chromosome in KS affects spermiogenesis. A recent transcriptome study of adult KS testes showed that many messenger RNA were differentially expressed in Sertoli and Leydig cells (183). It is still unclear whether these changes are merely changes that occur after the demise of normal testis architecture, with extensive fibrosis and hyalinization of the seminiferous tubules (98), that occur already in childhood (184), and perhaps already start *in utero*, and accelerate during puberty. Another recent study of fetal testes found a marked reduction in MAGE-A4-prespermatogonia by immunohistochemistry, and by transcriptome profiling of formalin-fixed, paraffin-embedded tissue, a large number of differentially expressed transcripts was found (185). The authors focus on the X chromosome PAR transcript, *AKAP17A*, and enrichment of long noncoding RNAs and speculate that the differential expression of these factors may perturb early gonocyte differentiation.

Until the late 1990s, the majority of men with KS were referred to treatment with donor semen. However, in a major part of men with KS, minor foci with production of small numbers of spermatozoa can be found (98, 180). Because spermatozoa from KS men originate in euploid germ cells (180), ICSI with these do not increase the risk of having a child with KS or other chromosomal abnormalities compared with using sperm from other men (186). The success rate in localization of such small sperm producing foci by random testicular biopsy or testicular sperm extraction (TESE) is low, and multiple biopsies may damage the testicular tissue (187).

"Future studies should aim at dissecting the pathogenesis behind autoimmune disease and KS."

Therefore, microdissection TESE (mTESE), where larger opaque, normal-calibrated seminiferous tubules suggested to contain spermatozoa are selectively removed by microscissors and the removed tissue examined for presence of sperm immediately, was developed (188). mTESE was found to be superior to conventional TESE in the only randomized, controlled study performed (189). The success rates in finding testicular sperm by mTESE are 44% to 66% depending on patient material, experience of the surgeon and laboratory technicians, and pretreatment of the patient with hCG or aromatase inhibitors before operation (190, 191). In selected KS cases, subcapsular orchiectomy could be considered (192). One might expect a higher chance of obtaining sperm when an entire testis is removed and systematically dissected in the laboratory. However, it seems necessary to improve the laboratory procedures before this method can be adopted. Circulating levels of FSH or inhibin B do not predict the chance of obtaining sperm in men with KS (192, 193). The testosterone level has been found to decrease following mTESE (194, 195) as well as

subcapsular orchiectomy (192). Because the majority of KS patients will benefit from testosterone therapy anyway, we consider the operative procedures to be ethically acceptable, taking into account the great wish of many couples to have their own child.

Increased age has been suggested to decrease sperm retrieval success rate. Therefore, testicular biopsies have been performed in teenage boys with the aim to cryopreserve testicular sperm. In the first early study, it was not possible to retrieve testicular sperm among seven nonmosaic KS boys 13.3 to 16 years of age (196), but testicular tissue was banked in the hope to develop sperm from spermatogonia in the future (197). In another European study, sperm were isolated in one of five boys aged 15 to 16.5 years old (198). Conversely, in one study, researchers were able to retrieve sperm in 7 of 10 boys 14 to 22 years of age (mean age: 15.5 years), after the boys were given testosterone replacement and aromatase inhibitor therapy for a period of 1 to 5 years before surgical sperm retrieval (199). Usually, testosterone replacement will have a negative influence on sperm production by a decrease of the intratesticular testosterone level, which is up to 400-fold higher than in the periphery and seems to be higher among KS males than controls (181), due to inhibition of the LH secretion by negative feedback mechanism. This must have been possible to avoid in the previously mentioned study by giving very modest testosterone doses (199). Furthermore, use of aromatase inhibitors for a long time may decrease the level of estradiol, which is an important modulator of bone structure. In a prospective study, the sperm retrieval rate (52%) in 25 nonmosaic KS boys 15 to 22 years of age was similar to sperm retrieval rate (62.5%) in 16 nonmosaic KS men 23 years of age or older (200).

In conclusion, the success rate in testicular sperm retrieval procedures is seemingly not improved by performing the procedure during adolescence. However, further studies will show if there is a place for cryopreservation of testicular tissue from KS adolescents with the aim of inducing sperm production from spermatogonia stem cells in the future. There are important ethical considerations in offering sperm retrieval and cryopreservation during adolescence or even younger, and it is not straightforward how to deal with these questions (201), although a Belgian study showed that parents of KS adolescents were in favor of such a possibility (202). We suggest that such procedures should, for now, only be performed within ethically approved protocols.

Children born of KS partners are generally healthy and without chromosomal abnormalities (179). Two exceptions are triplet pregnancies from Israel where a KS fetus was therapeutically aborted (203). However, if KS occur in 1:630 boys in the background population, a few cases with 47,XXY karyotype should be expected.

Data from animal studies

The KS mouse (41,XXY or 41,XX^{Y^*}) shows morphologically normal spermatogonia early in life, indicating normal proliferation and migration of primordial stem cells, but already from day 12.5, or even earlier, mitotic proliferation declined and led to eventual loss of germ cells (101, 102, 204), and as an adult, this mouse is quite similar to the human KS male, with small testes, small seminiferous tubulus containing Sertoli cells only, hyperplasia of Leydig cells, and the typical hypergonadotropic hypogonadism. Recent results in the 41,XX^{Y^*} mouse show that the spermatogonial stem cell pool is reduced fivefold already at birth, indicating that this process likely starts *in utero* (205), and this may be due to changes in their stem cell characteristics. Here, the authors showed decreased immunohistochemical expression of spermatogonial stem cell markers LIN28A and PGP9.5 and decreased messenger RNA expression of a number of factors, including LIN28A and regulating microRNAs (miRNAs), indicating a reduction of the stem cell niche already *in utero* (205). A summary of the results from the two different mouse models concerning the testicular function has recently been published (206).

Summary of best evidence

Transcriptome data of whole testes indicate a profound deregulation of the genetic machinery underlying normal spermatogenesis and steroidogenesis. The KS male is no longer considered infertile, because many can now benefit from mTESE.

Areas of controversy

The optimal age for mTESE has not been defined, and likewise it remains to be shown whether pretreatment with hCG or aromatase inhibitors will increase the yield of spermatozoa. Banking of spermatogonia in adolescents remains a controversial area with clear ethical problems. The seemingly inevitable demise of the KS testes has not been elucidated, and research within this area will be exciting in the years to come. We need genomic studies of individual cell types (*e.g.*, Leydig, Sertoli, spermatogonia, *etc.*) to tease out the exact temporal events underlying what has been coined "the testicular catastrophe" in KS.

Sexual function

There is a growing literature outlining an association between KS and sexual dysfunction. Among males referred to outpatient clinics due to sexual problems, an increased prevalence of KS of 1.7% was reported (207), indicating that sexual dysfunction may be more common in KS males compared with males from the background population. However, the existing studies do not reveal a clear picture. The self-reported degree of erectile dysfunction ranged from 2.5% to 23%, which was not significantly different from control men with infertility or sexual dysfunction (208–210). In contrast

to this, in the largest sample to date, KS patients reported significantly decreased erectile dysfunction when compared with males from the male background population (211). These findings could reflect sampling bias. Men seeking medical consultation due to infertility or sexual dysfunction may share the same prevalence of risk factors related to erectile dysfunction as men with KS. Comorbidity such as dyslipidemia, diabetes, hypertension, metabolic syndrome, and obesity is seen with a higher prevalence in men with erectile dysfunction (212) and in infertile men (213). Thus, the profile of comorbidity seen in men with KS correlates well with that seen in men with erectile dysfunction or infertility, and it correlates well with the reported increased prevalence of erectile dysfunction in KS males when compared with the male background population. Psychological factors such as depression, which is known to decrease erectile function as well as sexual desire (214), may also have an impact on the erectile dysfunction seen in KS.

Androgen deficiency can also be related to sexual dysfunction. Testosterone therapy has been reported to have an impact on sexual motivation and mood in men with KS (215) and hypogonadal men (216, 217). However, Yoshida *et al.* (208) did not find any significant difference in the incidence of sexual dysfunction including sexual desire between KS men with normal total testosterone level and KS men with decreased total testosterone level ($P = 0.58$). In support of this, Corona *et al.* (207) also did not find any significant difference in sexual dysfunction and sexual desire between KS males and controls when adjusted for total testosterone (Table 3).

When including all the existing studies, decreased sexual desire was reported by 10% to 61% of men with KS (207–209), with one study finding sexual desire to be significantly lower compared with controls (209), whereas others did not find any statistical difference between KS males and controls (207, 208).

Regarding premature and delayed ejaculation, perceived premature ejaculation was experienced by 9% to 65% of men with KS (207–209), however equal to or even significantly lower than in controls. Delayed ejaculation was experienced by 7% to 43% of KS men (207–209). As with erectile dysfunction, studies including subjects with sexual dysfunction or infertility found this prevalence to be nonsignificant (207–209). Conversely, when comparing KS males with the male background population, delayed ejaculation was experienced significantly more often in men with KS (211). Data regarding orgasmic function is also divergent, with one study finding that orgasmic function was reduced in 20% of KS males (208), although not significant when compared with infertile controls. A study by Skakkebæk *et al.* (211), on the other hand, found orgasmic function to be significantly decreased in KS men compared with male controls. Finally, testicular pain seems to be significantly more prevalent in men with KS, with a prevalence of 23% (211).

With respect to frequency of intercourse, no difference was seen between KS males and controls (207, 208, 215, 218), illustrating that, despite some degree of sexual dysfunction, men with KS are as sexually active as other males, irrespective of cohabitation status. However, KS males do have a significantly later sexual debut (211). We recently studied criminality among males with KS in a nationwide study and found that males with KS more frequently were convicted for sexual abuse offenses, in addition to burglary and arson offenses (219). The cause for this increased criminality, in which only a minority of KS males are involved, is likely multifactorial, with influences from executive function (EF) problems, delayed social development, auditory processing deficiencies, communication deficits, having been bullied earlier in life, social behavior and cognition problems, age-(in) appropriate sexual interactions, and poor decision-making skills (discussed later in the neurocognitive chapter).

There are a few observations of gender dysphoria and increased rates of bisexual and homosexual identity, which may suggest an increased frequency among KS males (211, 220, 221); however, currently, there is a gap in our knowledge concerning gender identity, gender dysphoria, and KS before any definitive conclusions can be made.

"Verbal deficits are among the most characteristic functional features of KS."

Summary of best evidence

The sexual dysfunction seen in men with KS most likely is an effect of their comorbidity rather than the syndrome itself, and evidence for an effect of testosterone therapy on sexual dysfunction is sparse.

Areas of controversy

More comprehensive studies including androgen status, data on comorbidity, and sexual function are needed to elucidate the level of sexual dysfunction in men with KS and the impact of comorbidity and androgens.

Neurocognition, Quality of Life, and Socioeconomic Aspects of KS

Neurocognition

Cognitive dysfunctions

KS is characterized by a number of deficits in cognitive functioning, including general cognitive abilities (*i.e.*, intelligence), language, and executive functioning.

Intelligence. KS males' overall full-scale IQ is typically lower than controls (222–225), averaging about 10 points (0.6 standard deviations) below that of the general population (58, 226, 227), but not so low as to constitute intellectual disability. This downward

Table 3. Sexual Function in Studies of KS

	Wu *et al.* (215)	Yoshida *et al.* (208)	Raboch *et al.* (218)	El *et al.* (209)	Corona *et al.* (207)	Skakkebæk *et al.* (211)
Design	Double-blind crossover with oral testosterone undecanoate	Cross-sectional case control	Cross-sectional case control	Cross-sectional case control	Cross-sectional case control	Cross-sectional case control
Participants (mean age ± SD) or [median (range)]	4 KS (35.3 ± 8.5)	40 KS (32.2 ± 4.0)	77 KS (31 ± 5.78)	53 KS [33 (26–40)]	23 KS (40.6 ± 12.3)	132 KS [41.7 (19.0–76.5)]
		55 controls (33.5 ± 4.2)	85 controls (32.7 ± 6.31)	75 controls [34 (30–39)]	1356 controls (51.7 ± 13.0)	313 controls [42.5 (17.0–77.2)]
Reason for medical consultation	Infertility	Infertility	Infertility	Infertility	Sexual dysfunction	KS recruited from endocrine, genetic, and fertility clinics
						Controls recruited from the male background population
Testosterone treatment	No treatment (minimum 8 wk)	NA	NA	NA	NA	95 KS received testosterone
						36 KS received no testosterone
						1 unknown
Sexual debut	—	—	—	—	—	Significantly later
Number of sex partners	—	—	—	—	—	NS
Frequency of intercourse/wk	4.5 (1.4)	Significantly increased: 4.4 (2.8)/mo	NS	—	NS	NS
Orgasmic function	—	Decreased in 20% of KS	—	—	—	Significantly decreased
Sexual desire	Frequency of sexual thoughts/wk: 2.5 (0.4)	Decreased in 10% of KS	—	Significantly lower (decreased in 55% of KS vs. 17% of C)	NS decreased in 61% of KS	NS
Erectile dysfunction		Increased in 2.5% of KS	—	NS (prevalence: KS 19%)	NS (prevalence of severe ED: KS 23%)	Significantly increased
Premature ejaculation	—	Prevalence of premature or delayed ejaculation: KS 57.5%	—	Significantly lower (prevalence: KS 23%)	NS (prevalence: KS 9.5%)	NS prevalence: KS 65%, C
Delayed ejaculation	—	Article I.	—	NS (prevalence: KS 7.5%)	NS (prevalence: KS 9.5%)	Significantly more often (prevalence: KS 43%)
Perceived ejaculation volume	—	Decreased in 42.5% of KS	—	—	Decreased in 62% of KS	—
Testicular pain	—	—	—	—	—	Significantly more often (prevalence: KS 23%)

Abbreviations: C, controls; ED, erectile dysfunction; NA, not applicable; NS, not significant.

shift in IQ among KS males predominantly reflects a deficit in verbal IQ rather than performance IQ, including nonverbal reasoning and spatial abilities, although the variability is large. This verbal IQ-performance IQ discrepancy among KS males typically emerges early in childhood (228, 229) and

sometimes dissipates in adulthood (230–232). This may reflect (delayed) development of more complex skills through learning and experience. Verbal deficits that persist into adulthood (*e.g.*, verbal memory and processing speed, lexical retrieval efficiency) may not be captured by earlier tests identifying more basic verbal dysfunction (*e.g.*, vocabulary) at younger ages. Finally, development of performance IQ may be halted as a function of hormonal and psychological tumult during puberty and/or accelerated deterioration of nonverbal processing later in life (*e.g.*, due to cardiovascular incidents).

Language. Verbal deficits are among the most characteristic functional features of KS, identified in 70% to 80% of KS males (22, 233). Language is also one of the broadest constructs relevant to KS, comprised of many interdependent elements, the results for which often differ across studies. Many researchers distinguish between *receptive* and *expressive* language functions (234–236) in their discussions of KS-related deficits. Receptive language skills involve the comprehension of linguistic stimuli and are largely based on perception and recognition capacities, whereas expressive skills are those involved in the production of linguistic content, with an emphasis on recall and vocal motor function. Receptive language deficits associated with KS include problems with auditory processing and semantic memory (225, 237), word decoding, and auditory discrimination and processing (238–240). In addition, KS males exhibit deficits in many expressive language skills such as speech onset (225) and articulation (241), word retrieval and verbal fluency (230, 232), and word formulation, as well as general expressive skills (239, 242).

Reading, writing, and literacy are also heavily affected in KS males (228, 242). Approximately 50% to 75% of both children and adults with KS demonstrate some level of dyslexia (232) compared with a 7% prevalence in the background population (243).

Typically, language deficits in KS emerge early in childhood (244) and persist into adolescence and adulthood (241). Although such KS-related language problems are consistent in the literature, they are not universal. The most prominent exception is a study that found no KS deficits relative to the general population in single-word decoding, spelling, receptive or expressive vocabulary, word retrieval, or verbal fluency (72). However, this lack of KS-related deficits may also reflect, at least in part, the complexity of the tasks themselves, for spelling, simple vocabulary and single-word decoding, word retrieval, and verbal fluency (*i.e.*, timed word retrieval) are all relatively simple, declarative skills, and there is consistent evidence that KS males' linguistic performance declines with increasing task complexity (225, 237, 239, 241). Bender *et al.* (218) found no KS-related decrements in single-word decoding, and Graham *et al.* (239) observed no KS deficits in any receptive language measures except syntactic comprehension.

Understanding and treating language deficits among KS males is critical for their well-being, for language is not only the foundation for one's understanding and communication with the world, but also essential for cognitive, emotional, and social development (242, 245). However, developing a comprehensive understanding of KS-related language problems poses a significant challenge, given the number and complexity of linguistic functions, many of which are related (*e.g.*, spelling and word decoding), nested (verbal and auditory memory), and/or relevant to either one or both forms (written/oral) and domains (receptive/expressive) of language.

EF. EF refers to cognitive control processes involved in goal-directed behavior and problem-solving, such as organization, planning, judgment, and decision-making, with specific functions that include focused and sustained attention, holding thoughts in working memory, inhibiting irrelevant information, and processing thoughts in a fluid and flexible way. KS deficits have typically been found for attention (72, 225, 238), inhibition (225, 246, 247), and both working memory and cognitive flexibility (225, 238, 248), although results from the relatively few EF studies among KS males are somewhat mixed (72, 247). The consistent verbal EF deficits among KS males suggest a potential linguistic cause for these performance decrements, but studies have found the diminished inhibition capacity of KS males to be independent of language skills and processing speed (246), illustrating the importance of controlling for intellectual, linguistic, and other potential confounds in KS research. In the most comprehensive study to date on EF among KS males, Skakkebæk *et al.* (249) used extensive regression and path analyses to control for and assess the independent effects of KS status, intelligence (IQ), personality, social skills, and testosterone treatment on the EF of 69 KS males and 69 matched controls. We found that the impact on EF of having KS was mediated separately by IQ and social skills, and this study stands as a model for future research to better understand the potential causes of, and treatments for, EF and other deficits suffered by those with KS (249).

Data from animal models. Both mouse models have, when tested, confirmed parts of the neurocognitive deficits seen in KS. In one study, 41,XXY mice showed delayed conditional learning in a Pavlovian approach procedure also testing memory (250), and in another study, 41,XX^{Y^*} mice were not able to solve a memory recognition task (204).

Summary of best evidence. Both human and animal studies show consistent deficits in EF, language, and intelligence among many males with KS, which interact with and affect social skills. It is clear that there

are complex interactions between these different domains of neurocognition.

Areas of controversy. It is uncertain how to best treat the neurocognitive deficits in men with KS. There is a definite need for large, well-designed intervention studies to improve neurocognitive skills in KS males.

Brain structure and physiology

Brain structure differences. A number of studies have investigated brain structure in KS males (251–259). The emerging picture is that KS males have smaller total brain volume, total gray matter volume, and total white matter volume compared with 46,XY males.

The ventral and central parts of the brain have been found to show a distinct pattern of large volumetric differences (251–259). The medial temporal lobes, including the hippocampi, bilaterally, have been found to be more affected, along with the insula and subcortical regions such as the striatum (Figs. 6 and 7). This pattern of differences to some extent mirrors that seen between 46,XX females and 46,XY males (260). Interestingly, the reverse pattern is observed in Turner syndrome (235), in that Turner syndrome females exhibit enlargements of the same areas, where KS males show decreased volume. Some of these effects may be due to epigenetic mechanisms. Support for this comes from the finding that KS males with skewed X chromosome inactivation have significantly smaller insula volume compared with KS males without skewed X chromosome inactivation (73) and display a general pattern of diminished gray matter volume in exactly the same regions, which are smaller in KS males than in 46,XY males (Figs. 6 and 7).

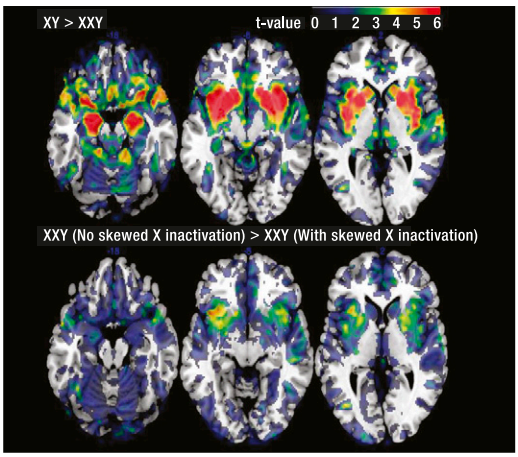


Figure 6. Bilateral ventral and subcortical gray matter volume in brain areas in KS. Bilateral ventral and subcortical gray matter volume in brain areas, including hippocampus, insula, and striatum, is found to be smaller in KS males relative to 46,XY males. A subset of KS males has skewed X chromosome inactivation. This group shows an exacerbated pattern of decreased regional gray matter volume. Figure shows unthresholded *t* maps for display purposes. Includes data from Skakkebaek *et al.* (73, 256).

The electroencephalogram. Early studies suggested that an increased proportion of KS males display slower α rhythms in their electroencephalogram (261, 262). This, however, has not always been replicated (263, 264), and the functional relevance of such a difference is uncertain.

The BOLD signal. One study investigated neural effects of a simple stimulus/response paradigm to investigate if KS males display a normal blood-oxygen-level-dependent (BOLD) signal, used as a marker of neural activation during functional magnetic resonance imaging (fMRI) (265). Given the endocrinological and physiological differences between 46,XY males and KS males, it might be expected that KS males would display abnormal nonspecific hemodynamic responses during fMRI. This, however, was found not to be the case. KS males as a group were found to have increased BOLD responses compared with healthy 46,XY males in and around the primary auditory cortices when listening to sounds (words). They were also found to display increased signal in the hand area of the motor cortex during responses to stimuli using finger presses. This effect was observed in the absence of a response time difference. In the visual cortex, however, no differences in BOLD responses were observed for visual stimuli (colored words). This suggests that the observed differences are not due to a system-level difference in hemodynamic responses.

The BOLD response has been found to increase with age in the normal population (266). The same was found to be the case in KS males, and no differences between KS males and 46,XY males were found (265).

Cognitive brain function

Very few functional neuroimaging studies have investigated the neurofunctional underpinnings of KS (235). Three main topics have been investigated, each targeting a field of cognition where KS persons display some level of deficit. The first topics are executive functioning and stimulus adaptation (265). In this study, which is also touched upon in "The Bold signal" previously, participants were given color word stimuli in either the auditory or the visual modality. The visual stimuli made up a simple Stroop paradigm, testing participants' EF. KS participants were found not to differ from 46,XY participants, neither in terms of their response time nor in terms of their Stroop effect, nor were they found to exhibit any differential brain activity during this task compared with 46,XY males. In the same study, an adaptation contrast was added by making one of the color words occur more often than the other. Both KS and 46,XY participants adapted their response times to the stimuli to an equal extent and displayed the same type of brain activation differences for frequent and infrequent stimuli (*i.e.*,

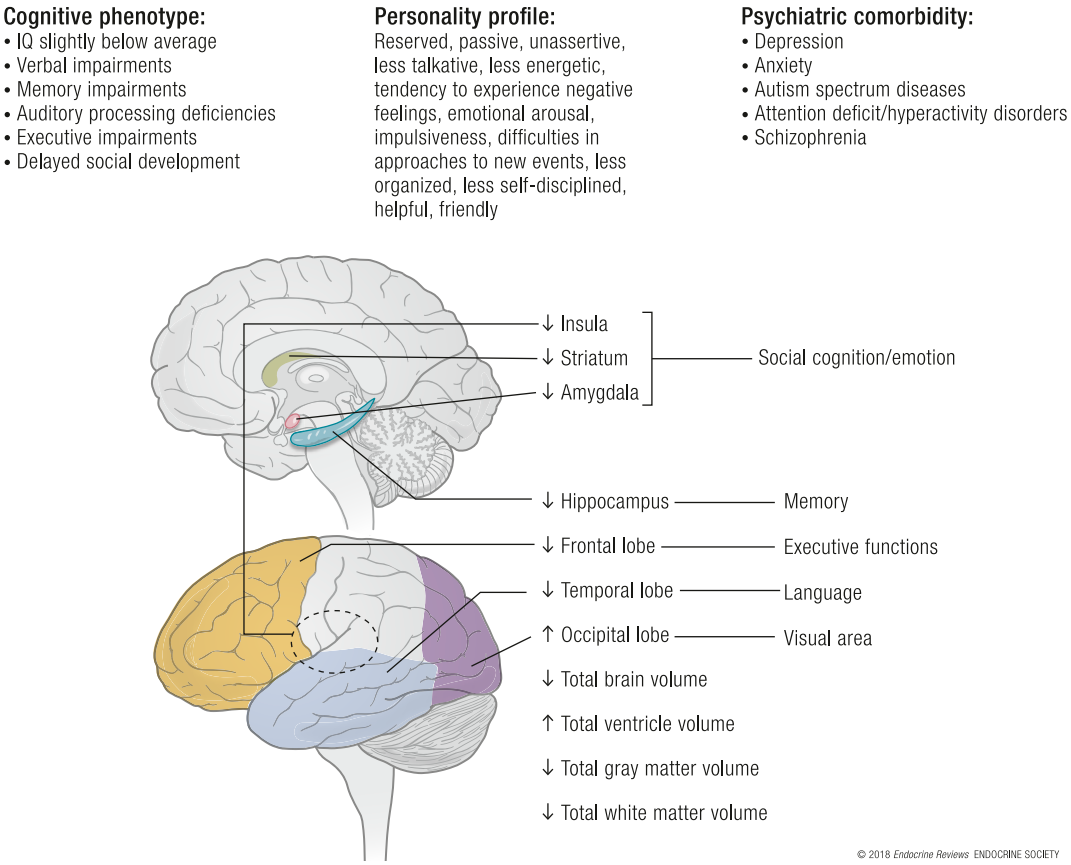


Figure 7. The figure depicts the current understanding of the cognitive phenotype, personality profile, and occurrence of psychiatric comorbidity in KS (upper part of the figure). In the lower part of the figure, the more consistent changes in size of certain brain regions in KS are depicted.

although KS males display learning disabilities, they seem to have normal adaptation to statistical properties of their perceptual input).

The second topic investigated with fMRI is language processing and lateralization (267). This study used language processing tasks and found that KS males have less language lateralization than XY males. The effects, however, were moderate and need to be replicated in a larger sample. In addition, lateralization indices were found, as it is often done, by comparing the number of activated voxels in a given region of interest across the two hemispheres. However, if KS males have greater signal in auditory cortex as found by Wallentin *et al.* (265), then they will tend to have more activated voxels. Activation levels may thus be just above threshold in KS males in, for example, the right hemisphere, whereas they are just below threshold in controls, resulting in a seemingly greater lateralization in the controls. We conducted an exploratory analysis of lateralization of raw regression coefficients in the temporal lobe (auditory cortex) in our study of single-word processing in a larger sample of KS males and found that it did not differ from that which was observed in 46,XY males (265). We did, however, find that 46,XY males had a significantly larger response to written words in the visual word form area. This effect may be related to the larger degree of dyslexia (see previous "Language" section) observed in KS.

A third topic is social cognition (268, 269). Elevated autistic traits have been observed in KS males (49, 270). One of the features of this is a decreased sensitivity to facial expressions. One study found KS males to display decreased activation in amygdala and the fusiform face area compared with controls (269), whereas another study found a tendency for the opposite effect in amygdala (268). In contrast, the latter study found that KS males relied more on prefrontal brain areas for processing of facial expressions (268). We have recently described generally poorer social skills and engagement among adult KS males and found that social skills are interlinked with EF deficits (249). More studies are needed to see if a consistent pattern emerges.

Summary of best evidence. There are consistent changes in the size of a number of brain regions on magnetic resonance imaging. Functional neuroimaging studies have described discrete changes in auditory and language processing, whereas findings regarding social cognition are equivocal, and tasks on executive functioning have been described to be similar to controls.

Areas of controversy. In KS, the interconnectivity between different brain regions has not been studied.

Functional studies are few and may not have been performed with appropriate design, and thus may not have targeted relevant neurocognitive abilities. Likewise, it is not clear if TRT affects functional neuroimaging.

Quality of life

Through the past decade, a few studies focusing on the impact of living with KS have emerged. These studies indicate that KS has important implications for the majority of these people, as children, as adolescents, and as adults. In the study by Turriff *et al.* (271) including 310 adolescents and adults with KS, 76% answered that having KS had significant negative consequence on their life. This is in line with the significant lower quality of life (QoL) reported in both boys and men with KS when compared with population normative data (272), a male reference group (273), or a male control group (211) or without a comparison group (104). In boys with KS, Close *et al.* (104) found that 67% of the boys reported adverse QoL with low scores on all subscales (physical, psychosocial, emotional, social). In addition, their data showed that total QoL, physical QoL, and social QoL were inversely associated with the severity of the physical phenotype and that 22% of the variance in total QoL could be explained by the physical phenotype (104).

Similar to the pattern in boys with KS, three studies in adult men with KS reported statistically significant decrements in all eight QoL subdomains (physical functioning, social functioning, role physical, role emotional, mental health, vitality, pain, general health) of the Short Form Health Survey (SF-36) (211, 273, 274). In addition, one of these studies also reported statistically significant decrements in all subscales of the abbreviated version of the WHO's QoL Assessment (physical health, psychological health, social relationships, environmental health) (211). Regarding predictors of QoL, the study by de Ronde *et al.* (273) demonstrated a positive and significant association between education and scores on the eight subscales of QoL, with multiple regression models finding a significant association between education and subscale vitality and general health.

Poorer sleep quality, with more sleep disturbance, high impact of poor sleep on daily functioning, and frequent use of sleep medication, was seen in a Norwegian questionnaire study (274). Here, poor sleep quality was a strong predictor of poorer QoL scores (274). In the study by Skakkebaek *et al.* (211), predictors of mental QoL and physical QoL were evaluated by structural equation models including both mental and physical subscales from the SF-36 and the abbreviated version of the WHO's QoL Assessment. In these models, lower mental QoL among men with KS were associated with lower income and living without a partner, whereas lower physical QoL was associated with less employment, lower income, daily medicine intake, and less physical activity (211). This study also found evidence that having KS is in itself associated with poorer life quality (211). In addition to the two previously mentioned studies among adults with KS, a third study has evaluated subjective well-being and other psychosocial outcomes in adults with KS (272). Their data showed that higher subjective well-being and psychosocial outcomes were associated with active employment status, increased social support, and a less severe phenotype (272). In another questionnaire study among adult Norwegian KS males, low scores on the SF-36 were also prevalent (274). These findings are further supported by the study by Turriff *et al.* (275), which provided further insight into the impact of living with KS using open-ended questions. The greatest challenges faced by adolescents and adults with KS were, according to their survey, infertility and psychological comorbidity, in addition to learning disabilities, physical phenotype, social relationships, employment problems, challenges with health care providers, and testosterone treatment challenges (275).

Whether testosterone has an impact on QoL has been investigated in two of the existing studies (104, 211). Close *et al.* (104) did not find any association between total testosterone level in blood and QoL for boys with KS, and Skakkebaek *et al.* (211) found no significant difference in QoL scores between treated and nontreated adult men with KS. However, both these studies were cross-sectional, and longitudinal studies are needed to properly evaluate the effect of testosterone substitution on QoL. Clinically, we see a clear benefit in most KS males from TRT with increased vigor, improved social functioning, decreased sleep length, and improved sexual function.

When KS males are asked about the level of satisfaction with health care services, the majority feel dissatisfied (211, 274), which is disconcerting. Many are followed by general practitioners or not seen at all in the health care system. This level of detachment from health care professionals may pertain to understanding the fundamentals of what KS is among patients, expectations to the health care system not being met, lack of understanding on behalf of the health care professional of the underpinnings of KS, or a mix of these issues. We believe that it calls for improved education among health care specialists, the need for a holistic approach to the KS patient, including a careful pedagogical approach in explaining how the syndrome affects individuals, and more research into the complex interactions between genotype, phenotype, and testosterone substitution in KS.

Summary of best evidence

The majority of boys and men with KS suffer from poorer mental and physical QoL, and with respect to predictors of QoL, phenotype severity/comorbidities and less social support/living without a partner among others may be associated with poorer QoL.

Areas of controversy

Only a minor degree of variance in QoL has been explained by the factors mentioned previously, indicating that other factors may have an impact on QoL in boys and men with KS. The influence of TRT has only been sparingly investigated.

Socioeconomic status

Socioeconomic measures, such as education, employment, and income, have long been found to impact people's general health and life expectancy across populations (276). From epidemiological studies, it is clear that educational attainment among KS males is considerably lower than the male population in general, and similar to males with 47,XYY syndrome (10, 64). It is also apparent that cohabitation and becoming a father (with donor semen or TESE) are much less frequent among KS males than other males (10, 64) and that employment rates are also lower, and in combination, these socioeconomic variables affect mortality negatively (10).

Questionnaires and clinical studies have shown that many KS males are underemployed, unemployed, or on disability pensions (211, 272, 274, 277). There is undoubtedly an influence from the increased and varied morbidity affecting many KS males (9) and their vocational attainment, which again forms an interplay with the ability to perform well in other areas, such as cohabitation and the chance of becoming a father. How testosterone substitution fits into this picture is not clear; however, in clinical practice, we encounter many KS males who suffer from musculoskeletal ailments, where low muscle mass, strength, and tone are likely forerunners. Therefore, we see a pressing need for longitudinal evaluation of the effect of testosterone therapy on a broad range of measures, also including measures of QoL and socioeconomy.

Summary of best evidence

The socioeconomic achievement of the average KS male is much poorer than the average male in society. It affects all aspects of socioeconomic status, including educational achievement, cohabitation, fatherhood, employment, and income.

Areas of controversy

It is not clear if earlier diagnosis can change the educational and thus the socioeconomic course for the average KS male and if early TRT can affect these parameters.

Hypogonadism and effects of testosterone supplementation

As stated, evidence of lower levels of testosterone, perhaps already during fetal life (30, 92, 98, 278, 279), neonatally (280, 281), and at least around midpuberty (46, 282), suggests that hypogonadism as well as the hypergonadotropic hypogonadism may have an impact on behavior and neurocognition in KS males, as sex hormones already from early fetal life are known to influence neurodevelopment and the brain (283–286). This raises the question of whether persons with KS may benefit from testosterone treatment early in life to improve their cognitive and social functions. Currently testosterone therapy is routinely administered to the majority of patients diagnosed with KS, usually starting around puberty or at the onset of hypogonadism later in life (Tables 4 and 5). Whether intervention with testosterone treatment at this age or even earlier may benefit patients with KS regarding cognitive and social functions has been investigated by very few studies.

In a retrospective study, Samango-Sprouse *et al.* (288) evaluated the effect of 3 months of early testosterone treatment (25 mg testosterone-enanthate) in infants (age between 4 and 15 months). They found evidence of a positive effect on cognitive domains at 3

Table 4. Testosterone Preparations Available for Treatment in KS and Suggested Doses for Adults

Substance	Brand Name (Company)	Format	Route of Administration	Suggested Dose
Testosterone	Tostran (Kyowa Kirin, Galashiels, United Kingdom)	Gel	Skin	20–70 mg per d
Testosterone	Testim (Ferring, Saint-Prex, Switzerland)	Gel	Skin	50 mg per d
Testosterone	Testogel (Bayer, Leverkusen, Germany)	Gel	Skin	50 mg per d
Testosterone undecanoate	Nebido (Bayer, Leverkusen, Germany)	Injection	Intramuscular	1000 mg every 10–14 wk
Testosterone undecanoate	Andriol (MSD, Kenilworth, NJ)	Capsule	Oral	120–160 mg per d in two to three doses
Testosterone enanthate	Testoviron (Bayer, Leverkusen, Germany)	Injection	Intramuscular	250 mg every 2–4 wk
Testosterone	Androderm (Allergan, Parsipanny, NJ)	Transdermal patch	Skin	2.5–7.5 mg per d
Testosterone	Axiron (Lilly, Indianapolis, IN)	Gel	Skin: axilla	30–60 mg per d

For children and adolescents, lower doses should be given (287). Some preparations are not available in all countries.

Table 5. Suggested Treatment and Intervention Strategies in KS

Treatment and Intervention Strategies
Childhood and Early Adolescence
Pedagogical supervision, including guidance on educational and lifestyle issues
Psychological referral if necessary
Information about support and peer groups
Puberty
Consider supplementation of testosterone
Pedagogical supervision, including guidance on educational and lifestyle issues
Psychological referral if necessary
Information about support and peer groups
Adulthood
Testosterone supplementation for most
Prevention of lifestyle diseases, including type 2 diabetes, obesity, chronic obstructive lung disease
Neurocognitive treatment
Fertility treatment
Estimation of bone density: DEXA scan
Information about the syndrome, including support and peer groups
Questions about well-being, physical activity, energy, sexual activity, libido

Abbreviation: DEXA, dual-energy X-ray absorptiometry.

and 6 years of age, including a positive effect on motor function at 6 years of age (288). At the ages of 9 to 11 years, a significant positive effect on social behavior was seen (289). In addition to these studies, a double-blinded, randomized study evaluating the effect of 2 years of low-dose oral androgen therapy (oxandrolone) in boys with KS aged 4 to 12 years reported improvements in visual-motor performance, social functions, and aspects of anxiety and depression after 24 months of treatment; however, no effect was seen on general cognitive function (125). Regarding testosterone treatment in adolescence, evidence for an impact of testosterone treatment on cognitive and social functions comes from two quite old observational studies both reporting a positive effect on learning, concentration, mood, and social function (290, 291), but no controlled trials exist in adolescent boys with KS. In contrast to these studies in boys and adolescents with KS, existing nonrandomized, cross-sectional studies of boys and adults with KS have found no effect of testosterone treatment nor testosterone concentration on cognitive, social, and motor functions (72, 249, 292, 293), except a single study reporting a positive effect of testosterone treatment on verbal fluency (258).

Although recently published studies in boys with KS have shown encouraging results regarding the effect of testosterone treatment on cognitive and social functions, randomized, controlled trials are indispensable to further elucidate the impact of testosterone therapy in infants, boys, adolescents, and adult men with KS and to identify the most optimal treatment protocol regarding testosterone preparation, dose, and age for initiating such therapy. In addition, these studies should also assess potential adverse effects of testosterone treatment.

Summary of best evidence

There is only scant evidence that TRT in infants and boys has positive effects on cognitive and social functions and no good evidence for this in adolescence or adulthood.

Areas of controversy

Better and larger long-term studies of the effect of TRT on social and neurocognitive functions are needed in all age groups. We recommend that randomized, controlled trials are indispensable to further elucidate the impact of TRT in infants, boys, adolescents, and adult men with KS and to identify the most optimal treatment protocol regarding testosterone preparation, dose, and age for initiating such therapy. In addition, these studies should also assess potential adverse effects of testosterone treatment.

Current State of the Art

Clinical care

As mentioned throughout this review, testosterone substitution therapy remains a cornerstone of proper treatment of males with KS. Although there are many unanswered questions concerning timing, dose, and route of administration, we recommend the initiation of TRT once the first signs of elevated LH and FSH occur to secure a proper masculine development of sexual characteristics during adolescence and to enable proper peak bone mass and muscle mass to prevent osteoporosis during old age. We discuss fertility issues before commencement of therapy, and postponement of testosterone therapy can be necessary if one wants to retrieve viable sperm at this stage. In pubertal KS boys, it has been reported that testosterone therapy increases energy and endurance and improves mood, concentration, and relations to others (291), and there is some evidence of increased psychosocial problems in periods without testosterone treatment in pubertal KS males (294). We argue for lifelong testosterone treatment to prevent lifestyle diseases such as osteoporosis, obesity, metabolic syndrome, and diabetes, although this practice is not evidence based. Treatment in a large group of young hypogonadal men of mixed origin (whereof some had KS) has been shown to have

a positive impact also on fat mass, muscle mass, and muscle strength, as well as sexual activity and related areas, and it improves positive aspects of mood (217). In older hypogonadal males, limited data suggest positive effects of treatment on visuospatial cognition and verbal memory (295). Some KS patients have normal testosterone values, but most have increased gonadotropin levels, an unfavorable body composition, and low hematocrit, indicative of a relative hypogonadism. Others with KS may not realize that they have typical hypogonad symptoms, and a trial period of treatment may show the benefits of treatment, using bivariate charts of testosterone vs. LH for proper dosage (100). In children and adolescents, dose escalation must be considered (287), starting with oral or transdermal treatment.

We aim to normalize LH and FSH during testosterone therapy and to avoid elevated hemoglobin and hematocrit, which is a common problem during treatment. Clinically, we note that about two-thirds of patients in our clinic prefer long-acting testosterone undecanoate or testosterone enanthate, and the remaining one-third of patients prefer testosterone gel, where brands enabling dose titration (Tostran; Kyowa Kirin, Galashiels, United Kingdom) are especially popular (Table 4). As stated, we need large observational, and preferably randomized and controlled, studies to answer questions related to efficacy and side effects through all phases of life. In particular, there is a scarcity of data on life with KS during middle age into senescence. Clinically, we see a mounting burden of comorbidity, which has rarely been documented in published research. We treat comorbidities according to consensus guidelines.

Concerning the many neurocognitive problems that males with KS can encounter, we are increasingly using neuropsychologists to provide neurocognitive therapy (Table 5).

Based on the published research to date, we urge for the creation of multidisciplinary clinics around the world and stress that care of KS males should take place in such units. This will ensure pervasive care from childhood through adolescence and into adulthood. We believe that centers around the world caring for KS males should implement policies to this end.

Perspectives

Males with KS face a bewildering array of medical, neurocognitive, and social problems, which are only beginning to become apparent in recent years. Clearly, there are complex interactions between genotype and phenotype, many of which we do not yet fully understand. We need to develop a more thorough understanding of the fundamental genetics and genomics of the syndrome to fully address the endocrine, neurocognitive, and cardiovascular disturbances. For example, is the testicular demise inevitable, or is there a possibility for rescuing testicular function, thus possibly avoiding infertility and the need for testosterone substitution? Why is it so difficult to diagnose KS? How detrimental is late diagnosis to the life of KS males? Would early diagnosis improve the lives of males with KS materially? It is clear that the current diagnostic approach is not sufficient, and we advocate for the incorporation of diagnostics of sex chromosome abnormalities, including KS, into neonatal screening programs. It is currently not clear which methodology would be most appropriate to use in a neonatal screening program, and therefore the costs of such an intervention is not yet clear.

Future research should also focus on delineating the complex interactions between the genotype and complex neurocognitive phenotype, both to understand the intricacies of the KS brain, and how the observed changes spell out clinically, and also to devise more efficient and effective treatment strategies. Thus, there is a need for much larger international collaborative efforts to study genotype-phenotype relations across all ages (we envision the inclusion of >10,000 KS males) and large epidemiological studies with merging of multiple registries to better delineate mortality, morbidity, medicinal use, and laboratory tests (level of testosterone, LH, hemoglobin, *etc.*), but also for intervention trials to study the effects of TRT and neurocognitive treatment.

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Abbreviations

BMD, bone mineral density; BMI, body mass index; BOLD, blood-oxygen-level-dependent; CI, confidence interval; cIMT, carotid intima-media thickness; CVD, cardio- and cerebrovascular disease; DVT, deep vein thrombosis; EF, executive function; fMRI, functional magnetic resonance imaging; FSH, follicle-stimulating hormone; hCG, human choriogonadotropin; HDL, high-density lipoprotein; HR, hazard ratio; ICSI, intracytoplasmic sperm injection; KS, Klinefelter syndrome; LDL, low-density lipoprotein; LH, luteinizing hormone; mTESE, microdissection testicular sperm extraction; PAI-1, plasminogen activator inhibitor-1; PE, pulmonary embolism; QoL, quality of life; SF-36, Short Form Health Survey; SMR, standardized mortality rate; TESE, testicular sperm extraction; TRT, testosterone replacement therapy; VTE, venous thrombosis; WHO, World Health Organization.