Endocrine Reviews, 2025, **46**, 447–478
https://doi.org/10.1210/endrev/bnaf005
Advance access publication 11 February 2025
Review

New Horizons in Klinefelter Syndrome: Current Evidence, Gaps, and Research Priorities

Angela K. Lucas-Herald,[1] Lise Aksglaede,[2,3] Ida Dyhr Caspersen,[2,3] S. Faisal Ahmed,[1] Francesco Carlomagno,[4] and Andrea M. Isidori[4,5]

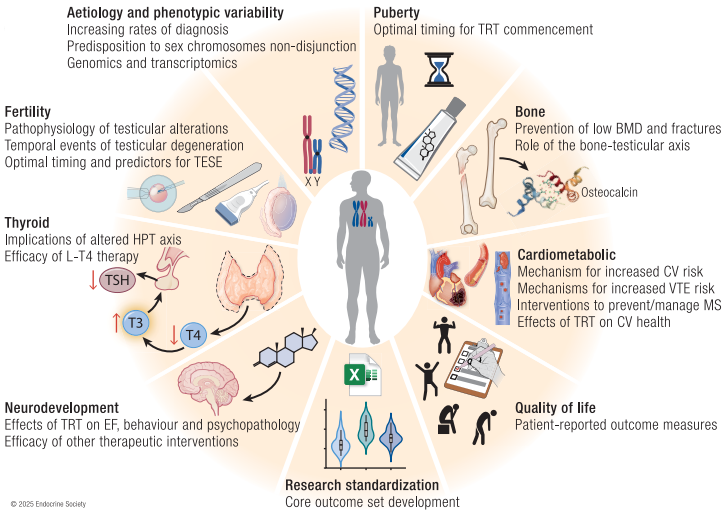
[1]Developmental Endocrinology Research Group, University of Glasgow, Glasgow G51 4TF, UK
[2]Department of Growth and Reproduction, Endo-ERN centre "Copenhagen University Hospital, Rigshospitalet," 2100 Copenhagen, Denmark
[3]International Centre for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health (EDMaRC), 2100 Copenhagen, Denmark
[4]Department of Experimental Medicine, Sapienza University of Rome, 00161 Rome, Italy
[5]Endocrine and Andrological Regional Rare Disease Centre—Endo-ERN centre "Azienda Ospedaliero-Universitaria Policlinico Umberto I," 00161 Rome, Italy

Correspondence: Andrea M. Isidori, MD, PhD, Department of Experimental Medicine, Sapienza University of Rome, Viale Regina Elena 324, 00161 Rome, Italy. Email: andrea.isidori@uniroma1.it.

Abstract

Klinefelter syndrome (KS) is caused by the presence of a supernumerary X chromosome (conferring the classical 47,XXY karyotype) and is the most common sex chromosome abnormality in men. The clinical features described in the early characterization of the syndrome include tall stature, small testes, hypogonadism, gynecomastia, and neurodevelopmental deficits. However, the syndrome presents a broad phenotypic spectrum that seems to be evolving, along with environmental and general health changes. Although a proportion of men with KS are asymptomatic, others experience numerous severe comorbidities, ranging from cardiovascular to autoimmune disorders. Once considered a hallmark of the syndrome, the inability to conceive can now be overcome with assisted reproductive technology. The neuropsychological stigmata, once overstated, thereafter inadvertently dismissed, now demand a more balanced and objective approach. Significant advances have been made in our understanding of KS over recent years, including the molecular machinery involved in the chromosomal disjunction that gives rise to the syndrome. Our understanding of the risk-benefit of testosterone replacement therapy has greatly improved; however, many gaps persist. Future work should be prioritized according to the needs of people with KS. There are opportunities for new research addressing the fields of fertility, cardiovascular prevention, neurodevelopment, quality of life, and bone health. Above all, solid registries and extensive prospective longitudinal studies are needed to enroll people with KS to determine their evolving needs as they progress through their lifespan. These studies would be best initiated with international collaboration to ensure the results apply to all those with this condition worldwide.

Graphical Abstract



Received: 27 February 2024. **Editorial Decision:** 7 February 2025. **Corrected and Typeset:** 24 March 2025

Key Words: Klinefelter, fertility, bone, testosterone, neurodevelopment, cardiovascular

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; ADMA, asymmetric dimethylarginine; AMH, anti-Müllerian hormone; AR, androgen receptor; ASD, autism spectrum disorders; BMD, bone mineral density; BMI, body mass index; cfT, calculated free testosterone; CO, crossing-over; COS, core outcome set; cTESE, conventional testicular sperm extraction; CVD, cardiovascular disease; DSB, double-strand break; DSD, disorders/differences of sex development; ED, erectile dysfunction; EF, executive function; EFT, epicardial fat thickness; FSH, follicle-stimulating hormone; fT3, free 3,5,3′-triiodothyronine; fT4, free thyroxine; HDL, high-density lipoprotein; HGA, high-grade aneuploidy; HPG, hypothalamus-pituitary-gonadal; HPT, hypothalamus-pituitary-thyroid; HR, hazard ratio; ICSI, intracytoplasmic sperm injection; INSL3, insulin-like factor 3; KS, Klinefelter syndrome; LC-MS/MS, liquid chromatography/tandem mass spectrometry; LDL, low-density lipoprotein; LH, luteinizing hormone; mTESE, microdissection testicular sperm extraction; OCN, osteocalcin; PAR, pseudoautosomal region; QoL, quality of life; SIR, standardized incidence ratio; SMR, standardized mortality ratio; SRR, sperm retrieval rate; T, testosterone; TESE, testicular sperm extraction; TRT, testosterone replacement therapy.

ESSENTIAL POINTS

- Klinefelter syndrome is a heterogeneous condition that can lead to several comorbidities throughout the lifespan, acting as a potent modifier of other individual and environmental risk factors.
- New opportunities for improving fertility and the general health outcomes in boys and men with KS are rapidly developing.
- Research gaps include better phenotyping, risk stratification, and understanding of the mechanisms sustaining the variability in the presentation of the syndrome.
- International research collaborations are needed to define the management of comorbidities, including the optimal timing and use of testosterone therapy.

Introduction to Klinefelter Syndrome

Klinefelter syndrome (KS) is caused by the presence of a supernumerary X chromosome (conferring the classical 47,XXY karyotype) and is the most common sex chromosome abnormality in men, with a prevalence of approximately 103/100 000 men (1). The clinical features first described by Klinefelter and colleagues in 1942 included tall stature, small testes, gynecomastia, long arms (a variable degree of eunuchoidism), infertility, and neurodevelopmental deficits (2). However, we now know there is a much broader phenotypic spectrum, which may explain why up to 50% to 75% of men with KS never receive the diagnosis (3, 4). For reasons that are only partially understood, some men with KS may appear largely asymptomatic, and others experience several comorbidities. Some patients with KS are identified due to more severe symptoms (eg, psychosocial, endocrinological, or cancer), while others, exhibiting a milder phenotype, are diagnosed during fertility evaluations. Consequently, research on KS is susceptible to ascertainment bias, and most studies must be understood within the context of this inherent bias risk.

Often, in teaching sessions about KS, the original black-and-white pictures are used to describe the condition; Fig. 1 shows recent photographs of the phenotypic variability characterizing adolescent and adult subjects with KS, highlighting differences in anthropometric measures, body fat distribution, gynecomastia, and genital appearance.

In the past, KS has been associated with tetrasomies and pentasomies of sex chromosomes with male phenotype, now termed high-grade aneuploidies (HGA), for example 48, XXYY, 48,XXXY, 49,XXXXY, etc (5-9). However, HGAs are exceedingly rare conditions, with a prevalence ranging from 1/18 000 to 1/100 000 or less (10). Even though patients with HGA share some of the features of KS, such as testicular dysfunction, they display additional specific facial features (such as hypertelorism with epicanthal folds, cubitus varus, clubfoot, pes planus, clinodactyly, and dental problems) and multiple musculoskeletal abnormalities (from radio-ulnar synostosis to scoliosis, hypotonic musculature, and tremors), as well as cardiac and renal congenital malformations (11-14). Severe cognitive and behavioral dysfunction and neurologic complications are also extremely frequent in people with HGA. Specifically with regard to testicular dysfunction, this may manifest at birth as impaired genital development, with micropenis and/or cryptorchidism, and gonadal damage may otherwise become apparent by pubertal age with pubertal arrest or, more frequently, delayed pubertal onset (15, 16). Testicular histology in people with HGAs frequently shows a more severe and diffuse condition of hyalinization and fibrosis of seminiferous tubules, loss of germ cells, and Leydig cells hyperplasia compared to classic KS (5, 17, 18). A worse clinical, hormonal, and metabolic phenotype is also typically apparent, and appears to be directly linked to a gene-dosage effect of the number of supernumerary X chromosomes (15). This review will focus on the nonmosaic 47, XXY KS, unless stated otherwise.

In recent years, interest in KS has grown substantially, with a 2.6-fold increase in related publications from 2000 to 2022 (60 vs 156 publications per year recorded on PubMed). Despite the interest, however, significant gaps remain in the overall understanding of KS, including mechanisms of complications, success of fertility preservation and assessment of quality of life (QoL) in affected individuals. This translates into differences in standards of care for children and men with this condition, with substantial variability in clinical practice among countries. In 2021, the European Academy of Andrology published international guidelines on the current standards of care for men with KS, endorsed by the European Society of Endocrinology (19), but the lack of high-grade evidence left several recommendations unsupported. As such, it is time to reconsider the current gaps and identify the research trajectories to prioritize in order to improve our current approach.

Current Evidence and Gaps in the Field of Genetics

XY Chromosomes Nondisjunction

KS arises from nondisjunction of the sex chromosomes, either paternal, occurring during the first meiotic division, or maternal, in the first or second meiotic division (20, 21). Different from whole-chromosome aneuploidies of the autosomes, sex-

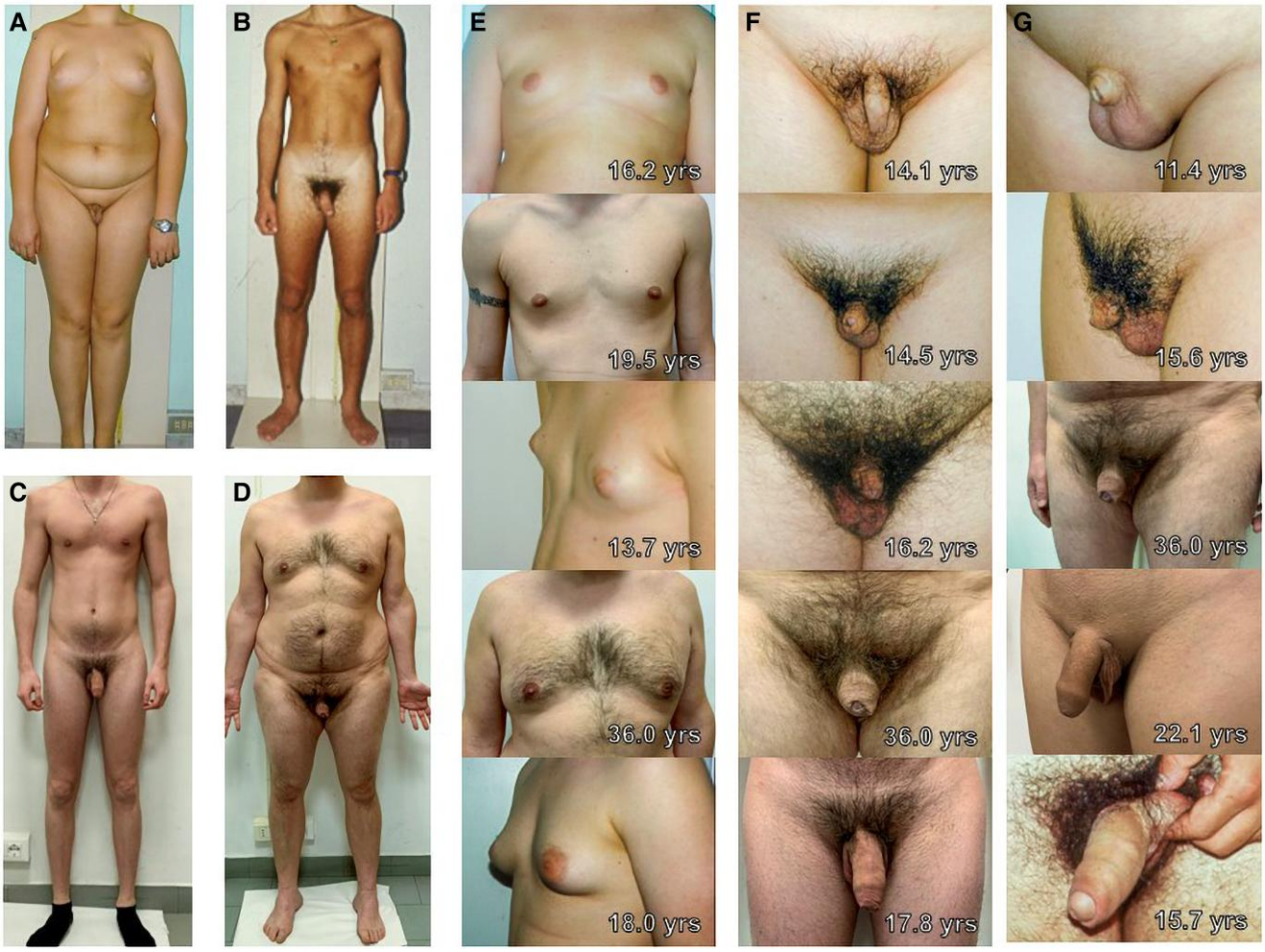


Figure 1. Phenotypic variability in anthropometry, body fat distribution, gynecomastia, and genital appearance in 12 adolescent and adult patients affected by 47,XXY KS. Panel A, Age 11.4 years, h 173.9 cm (+3.6 SDS), w 89.8 kg (+3.0 SDS), BMI 29.7 kg/m^2 (+2.2 SDS). Panel B, Age 15.7 years, h 167.2 cm (−0.8 SDS), w 58.4 kg (−0.6 SDS), BMI 20.9 kg/m^2 (−0.2 SDS). Panel C, Age 17.8 years, 183.0 cm (+1.1 SDS), w 68.5 kg (−0.1 SDS), BMI 20.0 kg/m^2 (−0.8 SDS). Panel D, Age 36 years, h 182.0 cm (+1.0 SDS), w 88.0 kg (+1.6 SDS), BMI 26.6 kg/m^2 (+1.3 SDS). Column labeled by E shows gynecomastia of varying severity, from absent to significant. Columns F and G show frontal and side views of external genitalia appearance, with reduced testicular volume, varying degree of pubic hair growth and the occasional finding of micropenis. Abbreviations: BMI, body mass index; h, height; KS, Klinefelter syndrome; SDS, standard deviation scores; w, weight. Note: Pictures acquired with prior written informed consent by the subjects (and the respective parents) for scientific medical use.

chromosome aneuploidies are frequently of paternal origin (22). In approximately 50% of KS subjects the supernumerary X chromosome is of paternal origin, and originates from a defective crossing-over (CO) between the X and Y chromosomes (20, 21, 23-28), preventing proper disjunction and segregation in daughter cells. Advanced maternal and possibly paternal age has been reported as a risk factor for KS (29, 30).

Although XY nondisjunction can be a stochastic event (31), sex-chromosome aneuploidy is more frequently reported in fathers of KS children compared to the general population (26, 32-34). Furthermore, although azoospermia is typically encountered in people with KS due to meiotic failure of aneuploid germ cells (35), spermatogenetic foci can be observed in the testes of some men with KS, showing a high frequency of sperm XY aneuploidy (36).

This evidence suggests a potential genetic predisposition to sex-chromosome nondisjunction. This could lead to a higher probability of fathering children with KS, possibly involving altered function of meiotic recombination genes. During meiosis, parental homologs recombine, allowing the shuffling of genome content and their alignment in the nucleus, and then synapse to form COs, required for chromosome segregation (37), allowing proper attachment of microtubules of the meiotic spindle to kinetochores (38). Recombination is initiated by the formation of a programmed wave of double-strand breaks (DSBs), which are then processed by DNA recombination factors assembling onto chromosome axes. A small subset of DSBs are then selected to become COs (39). Whereas autosomes rarely suffer from a lack of COs formation, as multiple DSBs are generated along their entire length (40), on the other hand, XY recombination is more complex since the 2 chromosomes are nonhomologous for most of their length and DSBs must form within a short region of homology: the pseudoautosomal region (PAR). Here, recombination must occur at each meiosis to form the so-called *obligatory CO*, which allows proper disjunction and segregation of XY in the daughter cells. In humans, XY nondisjunction in fathers with a KS progeny is associated with a reduction in recombination between XY (20, 21, 23-26, 36, 41). It is, therefore, expected that genetic alterations of genes involved in DSB formation and processing in

the PAR could cause XY mis-segregation, increasing the risk of aneuploid spermatozoa. This is indeed the case in mice, where the proper interplay of both α and β splice isoforms of Spo11, a type IV-A topoisomerase-like protein initiating DSBs formation, is required for appropriate XY recombination and synapsis, resulting otherwise in high rates of Y chromosome PAR DSBs formation and XY recombination failure, as well as production of sperm with sex chromosome aneuploidies (42). CO designation/maturation in the PAR must occur under a much more stringent control than in other chromosomes, as no more than 2 DSBs form in this region (43). Thus, it is conceivable that any variation in gene products involved in DSB formation and CO designation might primarily alter the formation of the "obligatory" CO in the PAR. However, these processes are neither dependent nor associated in KS with Y chromosome microdeletions, a common cause of spermatogenic defects (44). Future studies will need to investigate genes involved in DSB formation, those responsible for shaping the PAR structure, those engaged in COs designation and maturation, as well as those regarding the meiotic spindle assembly checkpoint. Individuals with XY pairing defects often also produce aneuploid sperm (20, 23, 25, 38, 45). For these reasons, spindle assembly checkpoint genes are another pool of genes of interest in the search for genetic causes of predisposition to XY aneuploidy in spermatozoa. Lastly, we acknowledge how epigenetic mechanisms might also contribute to XY nondisjunction, with a potential transgenerational inheritance in the offspring of KS men (46, 47).

X Chromosome Inactivation, Gene Escape and Overdosage, CAG Repeat Length of the AR Gene, and Parental Origin

X chromosome inactivation is a complex epigenetic process that occurs early during development in cells harboring more than 1 X chromosome. Thus, in 46,XX females, 1 of the 2 X chromosomes is randomly silenced on a cell-to-cell basis, although approximately 15% to 30% of X-linked genes escape inactivation and thereby contribute substantially to sex differences (48). Genes that escape inactivation are located throughout the entire X chromosome, but they predominate in the PAR1 of the sex chromosomes. Genes in the PAR1 are generally not inactivated, and therefore, men with KS have 3 active copies of these genes, which may explain some of the phenotypic traits. One example is the short-stature homeobox (*SHOX*) gene located in the PAR1 region. It plays a particularly relevant role in the growth and maturation of bones, and it has been shown to positively influence final height in KS (49). Furthermore, it has been speculated that overexpression of X-linked oncogenes may be responsible for the development of specific cancers (eg, hematologic cancers) (50). Other PAR1 and non-PAR1 genes have been reported to escape X inactivation in a model of induced pluripotent stem cells (iPSCs) generated from KS (and HGA) subjects, and their expression is directly related to the number of XY chromosomes (51). Furthermore, transcription factors have also been identified whose expression increases (ie, NRF1, ZFX, and TCFL5) or decreases (ie, CUX1 and CUX2) proportionally with X chromosome dosage (52). Skewed X inactivation, defined as ≥80% inactivation of the same copy of a gene, occurs in up to 50% of subjects with KS (53, 54) and, as such, has been proposed to contribute to the clinical phenotype of KS. Nevertheless, the evidence in this regard is weak, and most studies exclude an impact of skewed X chromosome inactivation on the phenotype (55-59). The parental origin of the supernumerary X chromosome (paternal vs maternal) has been suggested to impact the clinical phenotype. However, studies are controversial; most report no association (57, 59-61), whereas others report effects on anthropometry, pubertal onset and neurodevelopment (56, 62-64). The number of CAG repeats of the androgen receptor gene (*AR*) has also received attention, as they encode a varying number of polyglutamine proteins (from 11 to 35) that modify *AR* transactivation, thus determining androgen sensitivity (65-67), although some authors found CAG repeats to negatively correlate with some anthropometric measures, gynecomastia, testicular volume and bone-related parameters, other studies found no associations, also with regards to neurodevelopment or neuropsychological symptoms, vascular system, spermatogenesis, and QTc length (57, 58, 60, 68-71).

On a final note, although the present review focuses on nonmosaic 47,XXY KS, the role of mosaicism should be taken into account as a relevant contributor to phenotypic variability, as well as missed diagnoses. Specifically, subjects with (recognized) mosaic KS tend to present with a "more favorable" phenotype when compared with nonmosaic individuals, which may include larger testicular volumes, a lower prevalence and degree of hypogonadism, and a higher prevalence of sperm retrieval in semen analyses (72-74). On the other hand, it should be acknowledged how the notion of "pure" KS has been challenged by the evidence that men diagnosed with "pure" 47,XXY on standard karyotype analysis can reveal a significant degree of tissue mosaicism, a condition known as *hidden mosaicism*; in this phenomenon, which can be unveiled by an extended fluorescence in situ hybridization (FISH) evaluation of circulating lymphocytes (up to n = 500), epithelial cells from the buccal mucosa, and testicular tissue, revealing a 46,XY mosaicism rate up to ~40% among germ cells and Sertoli cells (75).

Omics—Genomics, Proteomics, Transcriptomics, Methylomics

The most recent genome-wide methylome and transcriptome analyses allowed the identification of alterations not only in the sex chromosomes but also in autosomal genes (76-80). Skakkebæk et al performed genome-wide DNA methylation profiling of leukocytes from 67 patients with KS and showed that the methylome and the transcriptome of both autosomes and the X chromosome are altered and that the profile is unique compared to both 46,XY and 46,XX individuals (76). Interestingly, the authors found a deregulation of neuron development, the immune system and Wnt-signaling. The latter has been linked to the development of cancer, malformations, bone density, diabetes, vascular disease, and cognitive deficits (76). Some of the characteristics of KS may be explained directly by an imbalance in genes on the sex chromosomes, but indeed, epigenetic factors which go far beyond the simple sex chromosomal gene-dosage theory have also been shown to play a role (81). Since epigenetic control has been linked to environmental conditioning, it is likely that where and how a person with KS is conceived and grows up has a significant impact in mitigating or exacerbating some features of the syndrome.

In summary, genome-wide alterations suggest that some candidate genes may explain the phenotypic variability

associated with KS; the altered dosage of genes disturbs genomic functions, potentially increasing the vulnerability to diseases, and epigenetics may be involved in the heterogeneous penetrance of some clinical features. However, the number of studies and, most importantly, the number of patients enrolled in these studies remains far too low. Harmonization in the methods and validation of external cohorts should be pursued, ideally involving an international consortium. Novel approaches include the investigation of circular RNAs, a group of noncoding RNAs regulating gene expression, which are differentially expressed among different tissues and might explain phenotype heterogeneity in sex chromosome aneuploidies comprising KS (82).

Current Evidence and Gaps in the Field of Endocrinology

Gonadal Function

One of the hallmarks of KS is progressive testicular failure with hypergonadotropic hypogonadism and infertility in adulthood. The degeneration of the testes has been shown to start in utero with a subtle reduction in the number of germ cells while retaining normal Leydig and Sertoli cells (83, 84). At the time of puberty, the degeneration accelerates with progressive loss of germ cells, hyalinization of the tubules, and Leydig cell hyperplasia (85), resulting in the classical histological description of the adult testis with extensive tubular hyalinization, Leydig cell hyperplasia within large nodules, and a heterogeneous pattern with Sertoli cell-only tubules, and in some patients few tubules with spermatogenesis (83).

Mini-puberty

Only a few studies of the function of the hypothalamic-pituitary-gonadal (HPG) axis in infants with KS exist, and the results are contradictory. The HPG axis is transiently activated during the first months of postnatal life in the so-called mini-puberty. In typically developing boys this activation starts with an early postnatal surge of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), followed by an increase in serum concentrations of testosterone (T) and insulin-like factor 3 (INSL3) with peaks at 1 month of age, further followed by increases in serum concentrations of inhibin B and anti-Müllerian hormone (AMH) peaking around 4 months of age (86). Then, a relatively quiescent period follows, lasting throughout childhood until the reactivation of the HPG axis in puberty. Mini-puberty is therefore considered a "window of opportunity" for the evaluation of the HPG axis, and it has been shown that serum T in typically developed boys during mini-puberty correlates with adult total sperm count (87); however, no studies on the correlation between hormone concentrations in mini-puberty and adult reproductive health in KS have been conducted so far.

In KS, T concentration during mini-puberty has been reported, ranging from low (88-91) to high-normal (92, 93) values compared to controls. In the most extensive study performed on infants with KS aged 17 to 152 days, T concentration measured by liquid chromatography/tandem mass spectrometry (LC-MS/MS) was significantly lower than controls, albeit with values still falling in the low-normal reference range (90, 91). INSL3 and LH were reported as normal for the age range, whereas FSH concentrations were significantly elevated despite normal inhibin B and AMH (90). Other studies have shown elevated LH and FSH despite high-normal concentrations of T and inhibin B (92, 93), which may indicate that infants with KS already exhibit some degree of compensated testicular impairment even at this early age. However, discrepancies in sample collection, storage, size, and characteristics, and more importantly, assay methods do not allow for drawing definitive conclusions.

Childhood

During childhood, and prior to pubertal age, KS is usually clinically silent, and the number of diagnoses is accordingly very scarce (3). Indeed, although some features may appear suspicious to the experienced clinician, such as tall stature, an increase in the fat/lean mass ratio, and mild speech, learning, behavioral, social and/or psychological difficulties, these are variable, nonspecific and typically go undiagnosed or may be inadvertently overlooked by the parents, teachers, and/or pediatricians of these children (19, 73).

The early studies on the HPG axis of prepubertal boys with KS described normal concentrations of T, INSL3, inhibin B, AMH, LH, and FSH, consistent with a similar axis pausing between affected and unaffected boys (see review (94)). However, hormone assays were less sensitive at that time, and the T levels were often below the detection limits. More recent studies suggest Leydig cell function can be found impaired even before puberty. Some studies reported reduced serum T concentrations (61, 95), but others found no difference (93, 95). Surprisingly, the 2 studies reporting a low T level found the LH concentrations normal (61) or paradoxically in the low-normal range (95). The concentrations of AMH, inhibin B, and FSH also differed between the studies. In the study by Spaziani et al, 145 boys with KS aged 0 to 12 years were divided into 3 groups according to age (93, 95). The investigators found elevated AMH and inhibin B but normal FSH in boys aged 6 months to 8 years, whereas, in 8- to 12-year-old prepubertal boys, none of the hormones differed from the controls (93, 95), whereas others found normal concentrations of serum AMH and no correlation between AMH and inhibin B, T, estradiol, LH, or FSH in prepubertal boys after the mini-puberty (96). By contrast, Davis et al found elevated AMH and FSH but normal inhibin B in boys aged 4 to 9.5 years (95), and Zeger et al found normal AMH, inhibin B, and FSH in all boys younger than 10 years (61). The finding of elevated AMH is of particular interest. It has been interpreted differently by the authors to indicate either higher sensitivity to FSH levels (increasing AMH secretion), or rather lower sensitivity of Sertoli cells to T (which is known to induce their differentiation) (93, 95, 97). The former proposal appears to be substantiated by the finding of a high correlation between inhibin B and AMH concentrations (93, 95). Alternatively, reduced intratesticular T concentrations have been hypothesized in early-pubertal KS boys, possibly persisting throughout pubertal development, which may determine a somewhat "slower" Sertoli cell differentiation, and thus a slower decline of AMH concentrations in this age stage. In the study by Aksglaede et al, the pubertal decline in AMH was postponed, which could indicate that boys with KS enter puberty later than expected (96). However, this was clinically not the case, and it was speculated that, since AMH is negatively regulated by T from the time of puberty, insufficient androgen action could also be an explanation for this delayed decline in AMH (98, 99).

Interestingly, in the studies showing reduced serum T concentrations, the boys presented significantly reduced penile length and testicular volume compared to controls (61, 95). By contrast, no such difference was found in the study showing normal T (93). The latter finding corroborates the hypothesis that in some boys compensatory mechanisms mitigated the effect of the supernumerary X, or, conversely, additional factors (epigenetic, autosomal and/or environmental in nature) aggravate its impact.

Puberty and transition age

In a recent semi-longitudinal study, including a detailed evaluation of 155 boys with 47,XXY KS during childhood, adolescence, transition age (defined as the period between completion of pubertal development and linear growth, and young adulthood, eg, 25 years of age (100)) and adulthood, the authors showed that testicular development progresses until Tanner stage 4, accompanied by increasing ultrasonographic testicular volume and rising serum concentrations of LH, FSH, and calculated free (cf)T (101). After that, Sertoli and Leydig cell functions become evidently altered, as documented by decreasing inhibin B/FSH and T/LH ratios. This progresses into pathologically high FSH and LH concentrations, a sharp decline in inhibin B, and a regression in testicular volume that accompanies qualitative (ie, hypoechoic lesions, microlithiasis) and quantitative (reduced echogenicity, inhomogeneity) changes at ultrasonographic examination (101). Specifically with regard to increased gonadotropin levels, these have been proposed as a guide for optimal timing of T replacement therapy (TRT) start in adolescents with KS when LH is >2 SD scores (SDS) according to age-related references, and may possibly also identify pubertal boys who should be tested for KS (19, 102). In childhood, inhibin B reflects the function of the Sertoli cells, whereas during puberty it becomes a marker of spermatogenesis, as the production of inhibin B is dependent on the presence of germ cells (103). A progressive decline in Sertoli cell function is also documented by AMH levels, normal until puberty, thereby declining to subnormal concentrations (96) approximating the peripubertal stage, paralleling the progressive seminiferous tubule hyalinization (104, 105). These results are consistent with previous findings placing the early detectability of gonadal failure at the peri-pubertal stage (88-90, 92, 103, 106, 107). However, data regarding the timing of pubertal onset are few and hardly comparable. A recent large study of 72 boys with KS seems to suggest no delay in progression to G2 compared to controls (106).

During transition age, FSH and LH concentrations plateau, and maximum concentrations of T and cfT can be observed. The T/LH ratio continues to decline (mostly due to a progressive increase in LH), indicating progressive Leydig cell dysfunction, whereas inhibin B continues declining toward nearly undetectable levels, indicating progressive Sertoli and germ cells impairment, accompanying the dramatic acceleration of seminiferous tubule degeneration in this stage (101, 103). Testicular volume, echogenicity, and echotexture remain relatively stable compared to the end of puberty. Nonetheless, the number of subjects with hypoechoic lesions and microlithiasis increases significantly; these are 2 significant predictors of worsening testicular function (101). Hypoechoic lesions in KS typically represent foci of Leydig cell hyperplasia (or Leydig micronodules) (108). Their formation mechanism is unknown; one hypothesis is that persistent high LH causes Leydig cell hyperstimulation (109).

Adulthood

Hypergonadotropic hypogonadism is typically present in virtually all adult males with KS. However, many subjects show total T levels falling within the reference ranges, albeit invariably associated with high LH concentrations. This condition is defined as *subclinical* or *compensated* hypogonadism (110, 111). During adulthood, testicular volume further regresses, accompanied by worsening echotexture and an increase in the prevalence of microlithiasis and hypoechoic lesions (101). T and cfT levels tend to decline after plateauing during transition age, with further worsening of the T/LH ratio and no other change in HPG axis hormones (101). As such, the prevalence of overt hypogonadism increases with patient age and has been reported to approximate between 50% and 63% to 70% after the age of 30 years (as defined by T levels <10.4 or <12.0 nmol/L, respectively) (72, 112, 113).

A microvascular explanation has been recently proposed for the development of hypogonadism in KS, stemming from a mouse KS model in which increased intratesticular T concentrations were found despite low serum T levels. The hypothesis of T entrapment in the mouse KS model was supported by the observation of a reduced testicular vascular bed and a defective T release in the bloodstream (114). These mouse alterations are progressively associated with reduced testicular perfusion dynamics, assessed by contrast-enhanced ultrasonography (115). A recent study confirmed slower testicular perfusion kinetics in men with KS (eugonadal and hypogonadal) compared to age-matched controls, which were associated with lower circulating T levels (116). Single-cell RNA sequencing of testicular cells from men with KS revealed gene expression indicating enhanced capillary endothelial cell activation, increased inflammatory crosstalk, disorganized vessel maturation and endothelial cell barrier permeability (117). In summary, a progressive vascular/inflammatory derangement in KS has been corroborated in different models and linked to advanced endocrine failure. Future studies will need to elucidate better the sequence of events, specifically whether the microvascular alterations in the testes of KS can be halted or reversed.

Bone Health and Metabolism

As the natural history of men with KS leads to the development of hypogonadism, implications on bone health and metabolism are expected (118, 119). Indeed, all fractures and osteoporotic fractures (hip, spine, and distal forearm) are more frequent in KS (120-122), and bone mineral density (BMD) is reduced. The prevalence of osteopenia (or low BMD) in adults with KS ranges from 21% to 44% (71, 123-125), whereas osteoporosis is from 9% to 13% (71, 124).

The alterations in bone mineralization could result from a suboptimal peak bone mass achievement, although studies during childhood, adolescence and transition age are scarce (125-127). A recent retrospective study on young individuals (~6 to 21 years of age) described a lower-for-age BMD and, more interestingly, a reduced bone mineral content adjusted for bone area, indicating "light bones" (128). Conversely, bone formation and resorption markers were not different in 71 adults with KS vs healthy controls. Still, half of the men with KS enrolled in the study were on TRT or had

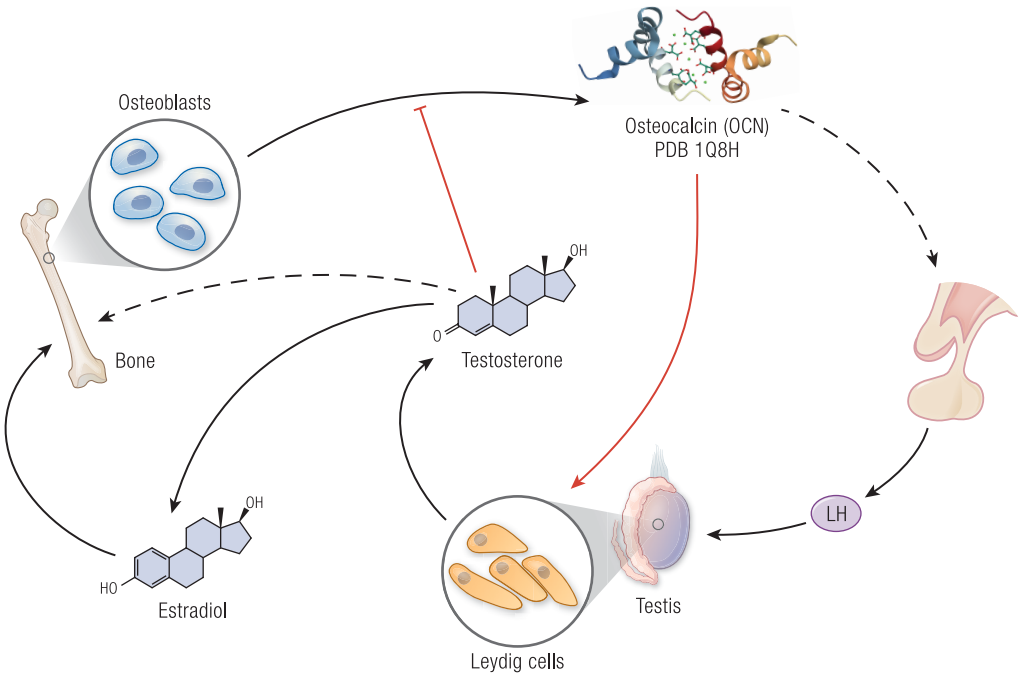


Figure 2. Bone-testicular-axis and the specific roles of osteocalcin and testosterone in the context of hypogonadism in subjects with KS.

Abbreviation: LH, luteinizing hormone. Solid lines represent established effects, dashed lines represent less significant effects, the red lines refer to novel reciprocal effects of testosterone and osteocalcin.

previously received T (123). No clear relationship exists between T levels and BMD (123) or between BMD and fractures (129), suggesting a multifactorial etiology. Interestingly, bone quality was also investigated through high-resolution-peripheral quantitative computed tomography (HR-pQCT), documenting altered microarchitecture, impacting both cortical and trabecular bone and reducing bone strength (130, 131).

Aside from T, other contributing factors include lower 25-hydroxy-vitamin D levels (123, 132), unfavorable body composition (123), reduced physical activity (133, 134), increased incidence of trauma (121), coexisting comorbidities, X chromosome inactivation and AR sensitivity (68). INSL3, a Leydig cell function marker, has also received attention for its role on osteoblasts and osteocytes exerted through *RXFP2* (135). INSL3 is not acutely regulated by the HPG axis, but its concentrations are linked to Leydig cell number (136), gradually declining with age (137). INSL3 induces osteoblast proliferation and differentiation alongside the production of osteonectin, osteocalcin (OCN), and bone alkaline phosphatase (135) and reduces the release of sclerostin (138). Thus, it appears to favor bone mineralization and the maintenance of bone mass (139). Although not significantly lower in infants with KS (90), INSL3 concentrations plateau from mid-puberty onward (140) and are reduced considerably in adulthood (141-144). INSL3 levels in men with KS show a positive correlation with total OCN, a negative association with sclerostin, and none with BMD (144, 145). The role of OCN is particularly relevant in consideration of the so-called *bone-testicular axis* (146-148). The proposed mechanism is a direct stimulatory role of OCN on Leydig cells, mediated by the GPRC6A receptor, to promote proliferation during development and testicular steroidogenesis (149), also able to stimulate *LHCGR* expression and, thus, LH signaling in Leydig cells (149). Furthermore, OCN has been shown to cross the blood-brain barrier and bind the GPR158 receptor, influencing central nervous system development and cognitive functions and possibly acting at a pituitary level (150-153).

Human studies have mostly confirmed the presence of a relationship between OCN and HPG axis hormones (T, estradiol, and LH concentrations) in various observational, cross-sectional, longitudinal, and population studies in boys and men from the general population (154-158). In a recent paper evaluating the bone-testicular axis in a large cohort (n = 254) of subjects with KS, total OCN values were shown to be significantly associated with gonadal status among men with KS, being highest in eugonadal and lowest among subjects undergoing TRT. Furthermore, after multiple adjustments for age and body mass index (BMI), total OCN concentrations are directly associated with LH and FSH values, indirectly with total and cfT, and decline after 3 months from TRT start (159). These results point to a negative effect of (exogenous) T on total OCN concentrations and highlight how higher total OCN concentrations indicate a higher degree of HPG stimulation and worse testicular function in men with KS. Figure 2 summarizes the perturbations of the bone-testicular axis in KS and the relationship to reduced T levels.

The hypergonadotropic state (high LH and high FSH) from late puberty onward has also been claimed as a possible contributor to the low bone mass of KS (139, 160). The evidence first emerged in women, where BMD loss correlates with increasing FSH years before estradiol drops (161, 162). Subsequent in vitro and preclinical studies have shown that FSH can act on osteoclasts via FSHR to promote bone resorption (163-165) and reduce osteoblast differentiation (166). On the other hand, clinical studies show conflicting results, supporting (167, 168) or disclaiming this mechanism (169). The aspect is particularly relevant when considering routes of TRT administration on bone. The transdermal route usually does not affect gonadotropin concentrations, as opposed to T injections, especially the long-acting T undecanoate, which

are more frequently associated with LH and FSH normalization or suppression. A 5-year retrospective study comparing men with KS by TRT route of administration found no differences in hip and spine BMD between the transdermal and the injection groups, despite the latter showing significantly lower, often suppressed, FSH concentrations (170); it should be noted however, that approximately one-third of men changed the route of administration during the observation period.

Even though reduced T in male hypogonadism usually results in reduced aromatization to estradiol (171), KS has been traditionally described as a condition of relative hyperestrogenism (in relation with T) (172), and estrogens are critical regulators of bone health in men, associated with both BMD and fracture risk (173, 174). Unfortunately, very limited evidence is available concerning the role of estradiol in KS (175, 176), mainly due to the difficulty of having reliable measurements, and especially with regards to the variable status of individuals with KS.

The start of TRT appears to elicit a beneficial effect only at the lumbar spine, the more "metabolic" site, according to a 2020 meta-analysis on adult men with KS; however, gonadal status, treatment duration or mosaicism could not be assessed (177). Data on bone quality (176) and microarchitecture (131) were also controversial.

Very recently, the double-blind, randomized, placebo-controlled TRAVERSE sub-trial on the effect of transdermal TRT on 5204 hypogonadal men (with baseline T levels <10.4 nmol/L) aged 45 to 80 years (178) revealed unexpected findings. The group receiving TRT experienced a 43% higher incidence of clinical fractures compared to placebo (3.50% vs 2.46%) after a median of ~3.2 years of follow-up. The most common fracture sites were ribs, wrists, and ankles. In contrast, the incidence of major osteoporotic fractures (hip, wrist, humerus, and clinical spine) was nonsignificantly different (20% higher in the TRT group). BMD and bone quality were not assessed, and the authors did not investigate physical activity and risk-taking. An accompanying editorial emphasizes that only speculations are possible regarding the mechanisms behind these results, including behavioral changes and engaging in higher-risk activities that might have occurred in men in the TRT arm (179), given its effect in boosting motivation and physical function in older men (179, 180).

In summary, there is an urgent need to address in prospective longitudinal studies: (i) the safety and efficacy of TRT on BMD and fractures, stratifying the risk by age at therapy start with specific focus on the critical window for bone mass accrual, route of administration, T and FSH target levels, different skeletal sites, and bone quality measures; (ii) the efficacy of bone-targeted therapies, starting from vitamin D and calcium supplementation (combined or not with TRT), and then of bisphosphonates, teriparatide, denosumab, etc. in osteoporotic men with KS; and (iii) whether persistently elevated FSH levels and/or reduced INSL3 concentrations may explain the limited efficacy of TRT alone on bone health in KS.

Thyroid Function and Structure

The first reports of thyroid function alterations in KS date back to the 1960s and 1970s and, strikingly, already hinted at a hypothalamic-pituitary-thyroid (HPT) axis dysfunction. A small case series comprising 5 men with KS first reported in the *New England Journal of Medicine* the presence of reduced ^{131}I uptake by the thyroid gland (181). Later, case reports from France (182) and Japan (183) emerged, pointing to thyrotropin deficiency and a blunted TSH response to TRH stimulation in men with KS. Subsequent small case series confirmed the blunted response to TRH (184), which was more apparent after TRT commencement (185). Together with other reports (186-188), the available evidence led to the interpretation of a "limited thyroid reserve" in KS (189), although not all agreed (190, 191).

More substantial evidence came from a Danish case-control study in 75 adults with KS compared to healthy controls, showing reduced free thyroxine (fT4) concentrations, with a resulting higher free 3,5,3′-triiodothyronine (fT3)/fT4 ratio (192), and no differences in TSH levels. The authors concluded there is an "inadequate hypothalamic-pituitary control of thyroid function in KS." Population studies from Denmark and England on 832 and 2208 men with KS, respectively (121, 193), found an overall higher prevalence of thyroid disorders, especially of autoimmune hypothyroidism. A higher prevalence of anti-thyroid antibodies in adults with KS has been reported by some (194) but not all (195), and it has been related to a general effect of sex hormones on autoimmunity (196).

Regarding the timing of these alterations, a single case-control study investigated 40 pubertal boys with KS compared to 157 healthy age-matched controls. The authors found reduced fT3 concentrations in KS, with no differences in fT4 or TSH levels. Thirteen boys also underwent TRH testing, with an altered response in 10 (197). The role of gonadal status on thyroid function was then investigated by an Italian multicenter case-control study in 174 KS vs 62 hypogonadal men on TRT, which also evidenced reduced fT4 levels (195).

On the background of these conflicting findings, a large retrospective longitudinal study was undertaken on 254 patients with KS throughout their lifespan, comprising eugonadal and hypogonadal (T-naïve) individuals as well as subjects on TRT (198). The patients were compared to non-KS age-matched cohorts with normal thyroid function, treated and untreated hypogonadism, and chronic lymphocytic thyroiditis. The results confirmed a higher prevalence of thyroid autoimmunity in individuals with KS of all ages, starting from prepubertal children. Individuals with KS also showed ultrasonographic signs of structural thyroid dysfunction, including reduced volume, lower echogenicity, and increased inhomogeneity compared to euthyroid controls. Lower thyroid hormone levels were present in KS from prepuberty to adulthood, whereas TSH concentrations were lower only in adults. The increased fT3/fT4 ratio, in the absence of altered peripheral sensitivity, suggests a dysfunctional HPT axis with an altered T4:TSH set point. As cfT was the only parameter correlated to thyroid function and appearance, a direct effect on thyroid hormone deiodination was hypothesized (198). In vitro testing demonstrated an inhibitory effect of T on pituitary deiodinase type 2 (D2) expression and activity, supporting the hypothesis that in male hypogonadism, the pituitary sensing of circulating thyroid hormones is altered (198). Figure 3 summarizes the proposed mechanisms associated with HPT axis dysfunction in subjects with KS.

In light of the above findings, a current gap lies in the clinical interpretation of thyroid test results in KS, which may change according to pubertal stage and gonadal status. To what extent the altered thyroid feedback contributes to the overall clinical picture of KS represents a research priority. Indeed, several comorbidities of KS, such as metabolic syndrome components, increased cardiovascular risk, cognitive

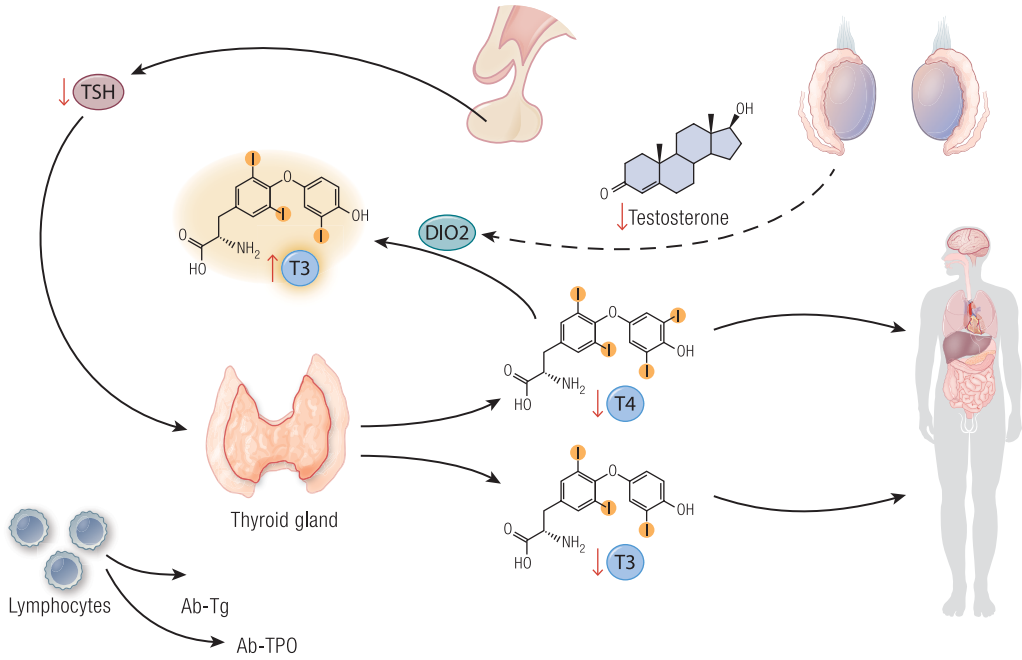


Figure 3. Hypothalamic-pituitary-thyroid axis and its perturbations in the context of hypogonadism in subjects with KS.

Abbreviations: Ab-Tg, anti-thyroglobulin antibodies; Ab-TPO, anti- thyroperoxidase antibodies; DIO2, pituitary type 2 deiodinase; T3, triiodothyronine; T4, thyroxine; TSH, thyroid stimulating hormone. Solid lines represent established effects, the dashed line represents the influence of reduced testosterone levels in hypogonadal subjects with KS.

alterations, reduced bone mass, and sexual dysfunction, are also common to hypothyroidism and might be at least partly related to reduced circulating thyroid hormones concentrations in KS. As such, we encourage a randomized clinical trial of levothyroxine therapy in individuals affected by KS, focusing on peripubertal boys and young adults, and targeting the above-mentioned outcomes, possibly related to the thyroid hormone action.

Current Evidence and Gaps in the Field of Reproduction and Sexual Function

Current Fertility Options and Novel Advances in Assisted Reproduction

Although a few men with KS have been reported to present with some degree of crypto/oligozoospermia, nonobstructive azoospermia represents one of the classic hallmarks of the syndrome (73). A number of pregnancies have also been reported following intracytoplasmic sperm injection (ICSI) from ejaculated sperm both in nonmosaic (199-210) and in mosaic cases (201, 211-215). Anecdotal reports of natural conceptions are also present in the literature (216, 217).

Two main hypotheses revolve around the production of spermatozoa by men with KS: a testicular micro-mosaicism, where patches of haploid 23,X/23,Y spermatogonia are present, or the capability by 24,XY spermatogonia to complete meiosis (218-220) successfully. A novel and intriguing hypothesis suggests that focal spermatogenesis in men with KS follows the loss of the additional X chromosome and XIST expression in a subset of mature (and euploid) Sertoli cells, which characterize type A seminiferous tubules (alongside euploid spermatogonia), and apparently occurs starting at puberty (221). If confirmed, these findings could suggest a risk of fathering a child with an abnormal X chromosome ploidy by men affected by KS to be similar to 46,XY men, as well as discourage referring prepubertal children affected by KS for testicular tissue cryopreservation for later transplantation.

Nowadays, minor foci of spermatogenesis can be found in the testes of many men with KS through conventional testicular sperm extraction (cTESE) (83, 219). Some have proposed using microdissection TESE (mTESE) to increase the retrieval rate by selecting larger tubules (222). The overall success rate of ICSI has been described to be reduced in the context of KS, compared with using sperm from other infertility etiologies in most (203, 223-225), but not all studies (226-228).

In 2018, a meta-analysis of 139 studies comprising 1248 men with KS who had undergone TESE, mTESE, or testicular sperm aspiration (TESA) (229), reported an overall sperm retrieval rate (SRR) per TESE cycle of 44%, with no significant differences between the outcomes of TESE vs mTESE. The overall pregnancy rate was 43%, with an identical live birth rate. No parameters could predict SRR, pregnancy rate, or live birth rate among age, testicular volume, LH, FSH, and total T concentrations. Similarly, a bilateral approach was not associated with higher success rates when compared to unilateral biopsy (229). However, a subsequent European multicenter, "real-life" study on 103 men with KS reported a much lower SRR of 21.4%, criticizing previous estimates as being "unrealistic and far from what physicians typically observe in the clinical practice" (230). Positive selection bias is relatively common in fertility studies and subsequent meta-analyses.

Regarding parameters capable of predicting successful retrieval via TESE, (bi)testicular volume, LH, FSH, total T, estradiol, sex hormone binding globulin, and prolactin

concentrations were not significantly associated with SRR (229, 231). In contrast, higher levels of both Sertoli cell function markers, namely inhibin B and especially AMH, were found in subjects with successful cTESE in a recent study on 119 nonmosaic men with KS, although with no clear cut-offs (231).

With regards to age, although some earlier studies had proposed that younger age at TESE might determine higher SRR (232-237), the above-cited meta-analysis and most, although not all (238), subsequent studies did not support this hypothesis (229, 231, 239). Nonetheless, different authors have strongly argued either in favor of fertility interventions, taking into account TESE as early as 12 years of age for fear of subsequent spermatogenic failure (240), or against it in any subject younger than 16 years of age for the lower SRR compared to values obtained later on (241). No advantage in earlier TESE has also been reported regarding retrieval of spermatogonia, which could be used to develop spermatozoa later in life, albeit the methodology remains only experimental (242, 243). Similarly, much discussion has focused on the role of TRT or other medical therapy concerning favorable or unfavorable TESE outcomes and on ethical aspects of early cryopreservation (244).

Nonetheless, as testicular damage in KS is progressive and reflects the natural history of the condition (101, 104), future studies will have to explore whether novel factors might predict successful sperm retrieval in these subjects, especially quantitative ultrasonography and texture analysis, as a clinical tool to monitor testicular parenchymal health and determine the most appropriate timing to schedule testicular biopsy (101).

Sexual Health and Gender Identity

Sexual function in KS has been the object of several studies. The prevalence of erectile dysfunction (ED) ranges between 2.5% and 23% (245-247), not dissimilar to the prevalence of ED in the general population (248) decreased sexual desire has been reported in 10% to 61% of men with KS (245-247). However, it must be taken into account that some studies relied on self-reporting by patients or on locally developed and nonvalidated questionnaires; as such, these results should be taken with caution.

In the most extensive available study (n = 132 subjects with KS vs 313 matched controls from the general population), investigators thoroughly assessed sexual function and quality of life (QoL) through surveys and validated questionnaires. Men with KS felt less confident as *a man* and *sexual partner*, experienced later sexual debut, erectile dysfunction, delayed ejaculation, and more frequently complained of testicular pain compared to controls (133).

The prevalence of perceived premature ejaculation has been reported to range from 9% to 65% of men with KS, whereas 7% to 43% of men complained of delayed ejaculation (245-247). Furthermore, no difference has been reported in men with KS with regards to the frequency of sexual intercourse, compared to controls (133, 245, 246), although age at first intercourse was slightly delayed (133).

Among factors contributing to sexual dysfunction in men affected by KS, a significant role is purportedly played by side effects of psychotropic medications (249, 250), with up to 80% of people treated with antidepressants (251), and approximately 50% of those receiving antipsychotics reporting sexual dysfunction (249, 250, 252-260). It is also possible that psychological factors, such as anxiety and depression, can contribute, for example, to worsen erectile function in KS men, as well as negatively impact sexual desire (see Section "Current Evidence and Gaps in the Field of Reproduction and Sexual Function") (261).

In the context of sexual dysfunction, TRT is expected to impact favorably on sexual desire and ED in hypogonadal men with KS, as circulating T levels are associated with sexual function in KS (262-264). TRT has been reported to ameliorate libido, mood, sexual activity (from flirting to sexual intercourse), and erectile function both in hypogonadal men (265-270) and in men with KS as well (271).

A few studies, including several case reports and small series, have described the incidence of gender dysphoria, or of related symptoms, in men with KS (or vice versa) (272-276). Among the larger studies investigating this topic, the results of the dsd-LIFE collaboration on ~200 men with KS reported a single subject who identified as "female" gender type, with no cases of gender change (277). In a recent systematic review comprising 11 cohort studies and over 1000 transgender individuals, the prevalence of 47,XXY was reported as 9/1013 (0.88%), higher than among the general population (278); however, the general KS underdiagnosis, coupled with potential selection bias linked to many transgender individuals undergoing karyotype analysis, limit the conclusions of this study.

It should also be taken into account how men with KS frequently experience body image dissatisfaction, particularly concerning the breasts (gynecomastia) and genitalia (low testicular volume), contributing to feeling "less masculine" (279, 280); the extent to which body dysphoria (or dissatisfaction) may contribute to gender dysphoria or dysphoric symptoms in subjects with KS remains a complex topic to be investigated.

Current Evidence and Gaps in the Field of Cardiovascular Medicine

Body Composition and Obesity

Although only a limited number of studies on body composition in children and adolescents with KS exist, it has consistently been shown that before puberty and even in early infancy, an unfavorable body composition with high body fat percentage and low lean body mass is present despite normal BMI (95, 126, 128, 281-283).

At 5 months of age, body fat percentage was significantly higher in 10 infants with KS as compared with 296 controls, whereas body fat percentage in another 10 infants treated with testosterone cypionate 25 mg intramuscularly monthly for 3 months did not differ from controls (281). In a recent double-blind, placebo-controlled trial, 93 boys with KS aged 4 to 12 years were randomized to oral oxandrolone 0.06 mg/kg/daily or placebo for 2 years (284). After multiple adjustments, treated boys showed a significantly lower percent body fat SDS compared to those receiving a placebo (0.29 vs 0.81). In prepubertal boys with KS (n = 89) aged 4 to 12.9 years, waist circumference was above the 90th percentile in 30% (282). In line with this, the waist-to-hip ratio was above the 95th percentile in 45% of prepubertal boys with KS aged 4 to 12 years (95). Based on whole-body dual-energy x-ray absorptiometry (DXA) scans, body fat percentage and the ratio between android and gynoid fat percentage

were significantly increased despite normal BMI in 62 boys and adolescents with KS aged 5.9 to 20.6 years (128). In that study, boys evaluated before and during TRT presented with a significant reduction in the ratio between android and gynoid fat percentages during TRT (128).

Adults with KS have an unfavorable body composition profile (80, 129, 285), and it has been shown that for any given BMI, men with KS have a higher truncal fat percentage as compared with controls, and truncal adiposity is the primary predictor of metabolic syndrome and insulin sensitivity in these patients (285).

Metabolism

In men with KS, mortality is increased (286), mainly secondary to cardiovascular disease (CVD) (standardized mortality ratio [SMR] 1.3; 95% CI 1.1-1.5) and diabetes mellitus (SMR 5.8; 95% CI 3.4-9.3) (120). Metabolic complications are common in men with KS, with reported rates of metabolic syndrome ranging from 34% (287) to 44% (285). More recent data from 376 men with KS on the Korean Endocrine Society Registry reported rates of hyperglycemia and dyslipidemia of 38.8% and 19.1%, respectively (288). A smaller recent cohort study of 30 young men (mean age 21.2 years) demonstrated increased levels of homeostasis model of insulin resistance (HOMA-IR) and TyG index (fasting triglycerides [mg/dL] × fasting blood glucose [mg/dL] / 2) as well as lower high-density lipoprotein (HDL) levels compared to healthy controls (289).

In children, metabolic syndrome is reported in boys with KS as young as 4 years of age, with increased waist circumference, low-density lipoprotein (LDL) cholesterol, and insulin resistance compared to age-matched control boys, despite similar overall BMI and in association with reduced reported exercise levels (282). In this group, the risk of metabolic syndrome was strongly correlated with inhibin B and AMH levels, and subjects with lower Sertoli cell markers demonstrated higher fasting blood glucose, fasting triglycerides, and LDL and lower HDL concentrations (95). Causality is not clear, but it may be that Sertoli cell function in childhood can be used as a marker for later CVD, with a prepubertal inhibin B < 50 ng/mL being most highly associated with metabolic syndrome (95). Overall rates of metabolic syndrome in the pediatric KS population range from 36% (282) to 80% (95). In addition, a population cohort study from PEDSnet reported that even after adjusting for covariates (obesity, TRT, and antipsychotic treatment), boys with KS had 70% greater odds of liver dysfunction (95% CI, 1.3-2.2) as well as increased rates of dyslipidemia and dysglycemia compared to age-matched controls (290). Of note, however, no differences in systolic blood pressure were detected (285, 290).

With regards to the effects of T therapy in childhood, in the randomized clinical trial mentioned above of oral oxandrolone in 93 prepubertal boys with KS, those in the treatment arm exhibited significantly lower triglycerides and lower HDL concentrations at 2 years compared to placebo (284).

Studies suggest that the development of type 2 diabetes in KS occurs from 20 years of age (291), although insulin resistance is reported in up to 24% of prepubertal boys with KS (282). It is worth noting that diabetes auto-antibodies, including GAD65 and IA2, are more frequent in men with KS (8% vs 1% in controls), which should also be considered in studies demonstrating hyperglycemia (292). In terms of management, the long-acting GLP-1 receptor agonist liraglutide and metformin have been shown to have beneficial effects in one study of hypogonadal men (comprising KS) aged 49 to 55 years with type 2 diabetes mellitus and obesity who presented with hypogonadism and ED (293). Men were initially given 2000 to 3000 mg/day of metformin and 12 weekly injections of 1000 mg intramuscular T undecanoate in association with a 1500-kcal Mediterranean diet and a 150-minute-a-week walking program for 12 months, followed by a further 12 months with the addition of 1.2 mg/day liraglutide. This resulted in improvements in weight, BMI, waist circumference, systolic blood pressure, glycated hemoglobin ($HbA1_C$), total and LDL cholesterol, and fasting blood glucose (293). To date, there have been no published randomized controlled trials assessing interventions to prevent metabolic syndrome in children and adolescents with KS or larger groups of men with KS. In addition, no studies have evaluated the impact of lifestyle modification.

Heart and Vascular Function

The incidence of congenital cardiovascular anomalies is increased in KS (SMR 7.3; 95% CI 2.4-17.1) (120) with reported cases of transposition of the great arteries, ventricular septal defects, aortic stenosis, tetralogy of Fallot, and hypoplastic left heart syndrome (294). In addition, extensive epidemiological studies have reported that men with KS have an increased risk of admission to hospital secondary to peripheral vascular disease (SMR 7.9; 95% CI 2.9-17.2) and intestinal vascular insufficiency (SMR 12.3; 95% CI 4.0-28.8) (285).

Noninvasive cardiovascular phenotyping can be done in several ways to assess for evidence of early subclinical vascular disease with methods including pulse wave velocity to measure arterial stiffness, carotid intima-media thickness for arterial plaques, flow-mediated dilatation for vascular endothelial function, and echocardiography for ventricular function (295). In one study of men with KS, left ventricular peak systolic velocity was reduced, with an association between myocardial systolic function, truncal body fat, and hypogonadism (296). Whether this is clinically significant and results in the development of heart failure is unclear, but it is likely given the fact that 30% to 40% of cases of heart failure are secondary to this phenotype (297). However, an Italian study of 74 men with KS demonstrated no difference in left ventricular systolic function but evidence of left ventricular diastolic dysfunction, increased intima-media thickness, reduced exercise capacity and maximum rate of oxygen delivery during exercise (VO_2 max) (298).

Plasma asymmetric dimethylarginine (ADMA) is a well-described marker of vascular endothelial function secondary to its ability to inhibit nitric oxide synthase, reducing overall nitric oxide and thus considered to play a pivotal role in the initiation and progression of atherosclerosis. In 2 studies, young men with KS have been shown to have higher levels of plasma ADMA compared to healthy age-matched controls (289, 299). Reduced levels of circulating endothelial progenitor cells have also been associated with atherosclerotic progression and cardiovascular morbidity and mortality, and their numbers have been reported to be reduced in men with KS, regardless of BMI or TRT (300). Epicardial adipose tissue,

as measured by epicardial fat thickness (EFT) on echocardiography, is another novel risk factor closely related to cardiovascular events, and a study of 221 men with KS and 77 age-matched controls demonstrated that men with KS had increased EFT (and truncal body fat) (301). In those who were not receiving TRT, EFT was similar to obese eugonadal controls, even though the BMI of the KS cohort was significantly lower (301). This suggests that the presence of an additional X chromosome in men may be a strong determinant of adiposity and risk of cardiovascular disease.

In healthy control men, there is a J-shaped association between T levels, as measured by mass spectroscopy, and CVD events, with men in the lowest and highest total T quintiles having an increased risk (302). Most men with KS receive TRT at some stage within their life course. One study has shown no difference in metabolic parameters in those receiving transdermal vs injectable T over a 5-year period (170). There was also no difference in those receiving TRT compared to those not (170). As such, it remains unclear what the effects of TRT at different ages and treatment regimens may be on cardiovascular risk (303).

A prospective international registry-based study may be best placed to establish the effects of TRT on metabolism and cardiovascular function via collaborative data collection on cardiovascular adverse events, blood pressure, lipids, and androgen levels, also in consideration of the recent findings from the TRAVERSE trial observing a higher incidence of atrial fibrillation and pulmonary embolism in hypogonadal men undergoing TRT (304). In addition, there is a need to review the impact of lifestyle on the cardiovascular risks identified in men with KS. A prospective study of 132 men with KS demonstrated reduced physical activity, reduced income, reduced social support, and increased daily smoking compared to controls, all of which have been linked to increased cardiometabolic risk (133)

Thromboembolism

Hospital admission data from Denmark have demonstrated that men with KS have up to a 6-fold increased risk of thromboembolic events compared to controls (285) with a resultant increase in thrombotic deaths (hazard ratio [HR] 1.76; 95% CI 1.18-2.62) and all-cause mortality following arterial thrombosis (HR 1.73; 95% CI 1.22-2.47) compared to controls (305). A higher prevalence of thrombophilic gene polymorphisms has been reported in a cohort of Egyptian men with KS (306). An earlier study investigating coagulation and thrombosis in KS identified a reduced endogenous thrombin potential in those receiving TRT, although with no difference in the procoagulant state (thrombin generation and coagulation inhibition) in 45 men with KS compared with controls (307). A subsequent study in 58 men with KS confirmed the presence of a procoagulant imbalance (increased thrombin generation and factor VIII concentrations) (308). TRT can have numerous effects on coagulation, including stimulation of coagulation inhibitors, antithrombin, protein S, protein C, and tissue factor pathway inhibitors (309). Studies have reported contrasting results thus far in terms of the effects of TRT, and again, it is not clear at what age the risk of venous thromboembolism starts to rise. This represents a critical research gap and one which should be prioritized due to the resultant risk of morbidity and mortality secondary to venous thromboembolism.

Current Evidence and Gaps in the Field of Cognition, Psychopathology, and Development

Clinicians treating boys and men with KS will acknowledge the complex psychological variability of individual resources, complaints, and needs. People with KS are as unique as everyone else. However, multiple developmental, cognitive, and psychiatric issues characterize KS on a group level, all of which may significantly affect life achievements and life satisfaction. Research elaborating on the neurocognitive phenotype of KS has intensified over the past 15 to 20 years, offering new and compelling insights into the challenges associated with KS. Primary focus areas of KS research include IQ, executive functioning (EF), and language, but promising newer studies have emerged on the nature of impaired social functioning and QoL in individuals with 47,XXY.

The existing literature on KS reflects the complexity of studying cognition and behaviors. Mental processes evolve throughout life, adapting to various factors such as genetics, hormonal changes, experience, environment, and aging. Designing research to describe this reality is challenging and between-study comparisons are further complicated by the heterogeneity of study designs. Methodology, participant characteristics (eg, age and pubertal status), as well as external factors (eg, time of diagnosis, T treatment, and parental factors such as psychiatric diagnosis, level of education, etc.) vary across studies and are not always addressed explicitly. Also, the issue of biased sampling is considerable: KS study participants may represent a more severely affected subgroup of the XXY population. This is of particular significance when considering findings on neurodevelopment in KS, because early diagnosis is typically associated with more evident clinical impairment. Taken together, multiple factors beyond the complexity of the 47,XXY phenotype affect current evidence on development, cognition, and psychopathology in KS (310-312).

Attention and Executive Functioning

One of the most fundamental aspects of human cognition is the ability to pay attention. Focused attention involves the capacity to concentrate on a particular target while ignoring distractors, and sustained attention is the ability to maintain this focus over extended periods by continuously allocating cognitive resources of vigilance (313). Attention deficits are consistently reported in the KS literature. Most studies employ continued performance tasks, measuring performance stability in reaction time and target sensitivity. Ross et al (314) described deficits of sustained attention characterized by prolonged and unstable reaction time and lapses of attention (omissions or target misses). Findings of impaired attention have been replicated in later continued performance task studies (310, 315-317), with effect sizes of attention deficits exceeding those of executive dysfunction (316, 318). This makes failures of sustained attention a likely key-constraint in the impaired higher-order executive skills of XXY. Moreover, a potential age-related effect of attention deficits in KS has been described (59): younger boys (4-9 years old) showed a reduced capacity to sustain attention that seemed to normalize with age (ie, boys with KS aged 10-18 performed at the level of the normative sample). Symptoms of inattention are also prevalent when assessing everyday behaviors of boys with KS using rating scales (311, 316, 318), and symptoms of inattention are apparent even in toddlerhood (319).

At the higher-order level of attention control, impairments of executive function (EF, ie, self-regulatory and control processes) are common in KS. However, the severity of deficits varies substantially across studies. Cognitive tests of individuals with KS show reduced ability to inhibit (311, 315, 320, 321), although exceptions exist (59, 316). In addition, studies describe reduced capacity of working memory (315, 316, 322, 323), weakened mental flexibility (310, 312, 315, 316, 324, 325), and impaired problem-solving and planning skills (316).

While EF performance tests help predict academic achievement (326), behavioral ratings possess the best ecological validity (327) and may provide additional insights into the everyday self-regulation of individuals with KS. So far, only a few studies have included such ratings. Substantial deficits across multiple EF subcomponents such as cognitive and behavioral control are reported (315, 318, 328) and precursors of executive dysfunction are manifest from early childhood (329). However, EF impairments in childhood may be subtle (only slightly >1 SD from the normative mean) and become more evident with age (330), possibly because of increasing expectations for self-regulation.

Consistent with the evidence presented above, attention deficit/hyperactivity disorder (ADHD) is common in XXY, with an average of 43% of children and adolescents fulfilling diagnostic criteria (331). Compared to the global prevalence rate of around 5% to 7% among children and adolescents in the general population (332, 333), individuals with KS face a greatly increased risk of being affected by ADHD symptomatology and related comorbidity (334, 335). The predominantly inattentive subtype is the most prevalent (311, 316, 336) and comorbid ADHD worsens performance and ratings of EF in subjects with KS (337).

Abilities related to sustained attention and EF are essential for adaptive and goal-oriented functioning in everyday life (338), and improving self-regulation could have vast beneficial effects for people living with KS. Although EF skills depend heavily on verbal support (339) and verbal ability is a well-known area of impairment in KS, verbal abilities and EF deficits in KS have not been successfully linked (316, 321). Learning more about the factors supporting executive dysfunction in KS will significantly advance our chances of alleviating these impairments. Recently, a neural basis for executive dysfunction in KS was identified, and the severity of EF impairments linked to the characteristic atypical pubertal development (317). Yet, the effects of TRT or psychostimulants like methylphenidate on sustained attention, initiation, impulsivity, drive and, ultimately, behavioral control in KS are largely unknown and need to be investigated. However, preliminary data suggest a positive effect of hormonal replacement therapy on executive functioning in KS (340) Lastly, the extent of shared features of ADHD between 47,XXY boys and 46,XX girls is unclear. As ADHD in girls is often dominated by inattentive symptoms and internalizing behaviors that are easily overlooked in a diagnostic context (341), clinical guidelines for KS may benefit from this area being specified.

Language, IQ, Learning Disorders, and Achievement

Language

Language impairment is among the most consistent findings in the KS literature, affecting an estimated 70% to 80% of boys with KS (331). Language plays a crucial role in supporting cognition across social, emotional, academic, and self-regulatory domains, and studying language in KS is essential to understand, treat, and, if possible, reduce challenges in these areas.

XXY-specific delays in language development are evident from toddlerhood at the earliest levels of verbal expression, with reduced vocabulary size and number of utterances (342-345). For school-aged boys and adolescents, findings include deficits of basic language abilities such as receptive and expressive vocabulary (314), phonological processing (346, 347), and impaired structural language, for example, processing of grammar (346) and production of syntax (348). However, more substantial difficulties are identified at complex language processing and expression levels, particularly within pragmatic language, such as understanding and using language in context (59, 314, 344, 346). Verbal problems seem to persist into adulthood (349, 350). Many studies include both parent reports and assessments and effect sizes are large, reflecting the substantial clinical impact of language dysfunction (351). Language disorders may be the most prevalent developmental challenge in KS (73, 335), with many boys with KS needing speech and language therapy (352-354). However, taking the high prevalence of autism spectrum symptomatology into account (331), it remains unclear how "pure" and specific the language disorders of KS really are. Language impairments and autism symptoms are reported to significantly overlap in samples of children, adolescents, and adults with KS (59, 336, 355). To clarify potential subgroups of diverse etiological causes of XXY language impairments, deficits need to be established in the presence of intact social interaction and nonverbal communication, like the intensified use of compensating pointing gestures (345).

IQ, learning disorders, adaptive functioning, and achievement

Like the language domain, intellectual abilities in KS have received extensive interest. Although full-scale IQ typically falls within the lower range of average performance (displaced downward with a mean of 10 points or 0.7 SD) (318, 343, 344, 356), intellectual disability is not common (331). Findings persist when comparing KS performance to siblings or controls matched on social class (354). The variability of intellectual capacity is substantial, ranging from extremely low to superior (335, 336, 351, 354). Profiles typically include greater difficulties in verbal domains (vocabulary, verbal comprehension and reasoning) compared to nonverbal/performance IQ (visuospatial reasoning and abstract thinking) (59, 314, 335, 336, 357), with medium-to-large effect sizes for verbal IQ and small-to-large effect sizes for nonverbal IQ (344). However, relatively superior nonverbal skills may dominate a subgroup of individuals with KS (358), as may profiles of balanced intellectual abilities (343).

The developmental trajectory of intellectual functioning in KS is not clear. Verbal IQ is affected from childhood but may normalize with age (325, 349, 359). However, the opposite direction may also hold (59). Although the prevalence of specific learning disabilities varies across samples, dyslexia and specific reading disability are very common, with rates up to 75% (170, 354, 356, 360-363). Interestingly, new preliminary data suggest prenatal diagnosis and early T therapy may positively affect reading abilities (364). Specific deficits of mathematical skills are also observed (343, 354), but generalized, not specific, learning disabilities may best describe most boys and adolescents with KS (359). Moreover, a

familial predisposition to learning disorders increases the vulnerability of disability and explains part of the higher prevalence in KS (365).

On tests of achievement, boys with KS perform below the level of typically developing peers in numeracy and literacy (328, 357, 359). By the time they finish high school, adolescents with KS have, on average, fallen 4 to 5 grade levels behind and are more likely to have received special education than unaffected siblings (359). In other words, KS may significantly impact and slow the rate at which boys and adolescents reach academic milestones, ultimately affecting socioeconomic attainments of employment and income levels (133, 366).

Adaptive functioning in KS is of interest because it goes beyond IQ in describing overall life skills, that is, the ability to integrate cognitive capacities and independently navigate everyday challenges. To date, only a few studies have addressed adaptive functioning in KS, all including minors or young adults (up to the age of 20). In a sample of 57 boys and adolescents with 47,XXY, more than 30% presented with moderate to severe impairments of adaptive behaviors (318). More specifically, children with KS seem to adjust significantly worse in contexts with social and communicative demands (328), with adaptive scores in the low end of the normal range (328, 367).

A recent study by Samango-Sprouse et al (2020) on prenatally diagnosed boys with KS suggests potential beneficial effects of early hormonal therapy on the developmental curves of baby and toddler problem-solving skills, language, and motor function (368). Although large cross-sectional studies like this have generated important insights, longitudinally designed investigations are needed to determine whether IQ, learning disorders, and adaptive behaviors in KS are best understood in terms of late maturation or by the principles of "growing into deficits" (369). Understanding this question will be helpful to clinicians guiding families and their network on how to best support a child with KS and what to expect prognostically.

Social Cognition and Functioning

Boys and men with KS are often described as unassertive, shy, passive, and socially withdrawn (344, 354, 370). Despite the inherent complexity of social cognition and adaptation, recent studies have attempted to specify perceptual, cognitive, and emotional mechanisms underlying social behaviors in XXY. Stable findings include the ability to read the mind of others. Individuals with KS are less skilled at identifying emotions from facial expressions and resonating emotionally with others (ie, to demonstrate empathy and mentalizing abilities) (310, 355, 371, 372). Impaired processing of facial affect reaches clinical significance in up to 43% of children and 33% of adults with KS (331). Difficulties in decoding emotions from tone of voice suggest XXY affect recognition deficits to be multimodal (373). In addition, an impact of social load on cognitive processing in KS has been described. As social load intensifies, information processing becomes more inefficient and inaccurate, possibly reflecting deficits of higher-order classification and interpretation of social information (374). In a recent study, the effects of social load on interactive behavior was studied in young children with KS: the children displayed reduced ability to imitate play and to initiate, maintain and endure social contact compared to typically developing peers, and impairments were more pronounced as social load intensified (375). The findings on emotion recognition, social load, empathy, and mentalizing are independent of IQ, age, and language ability (351, 355, 374) (374). Social information processing in XXY has been further explored by means of biometrics such as eye-tracking and heart rate measures. Individuals with KS demonstrate atypical and (in terms of decoding emotional states of others) inefficient fixation patterns, with less initial and less total fixation at the eye region (376). This tendency of attending less to the eye region during social interactions is present at early stages of development in children with KS and is associated with delayed language abilities in 1-year-olds with KS (377).

Although the diagnostic prevalence of autism spectrum disorders (ASD) in 47,XXY is substantially higher compared to the background population (on average, 18% vs 0.6% in the general population (331, 334), with symptoms present already from early childhood (378), subdiagnostic symptoms are even more commonly observed, corresponding to an average frequency of 25% in children with KS (331), and ranging from mildly to severely impairing (311, 318, 335, 336, 346, 355, 361, 378-380). Bouw et al (378) identified joint attention, namely, modulated verbal or nonverbal communication about a shared focus, to be a precursor of ASD in toddlers with KS. Other studies have suggested reduced mental flexibility to explain ASD behaviors in KS (312, 381).

The prevalent subdiagnostic expression of social impairment in KS may arise from subtle, but important features that are somewhat atypical to the ASD continuum. Social motivation, a key construct thought to contribute to a developmental cascade of social deficits in ASD (382) seems to be intact in children with KS (336), as is social awareness (318). Also, KS has been associated with increased social anxiety compared to ASD and nonclinical controls (371, 380), indicating an intact capacity of individuals with KS to reflect on themselves in a social way, combined with reduced ability to decode and act on social information. Although not consistent (375), these findings might guide therapeutic interventions targeted at improving social skills and well-being of individuals with KS. Preliminary research supports a beneficial effect of social management training on behaviors essential to social adaptation, that is, impulse control and self-reflection in men with KS (383). As social contexts provide unique opportunities for experience-based learning, enabling social participation will potentially improve long-term developmental outcomes in XXY. Although challenging to document in a research context, the effects of therapeutic interventions are of major interest to the field. Ratings of social anxiety, self-esteem, and QoL may be useful in this endeavor.

Disorders of Emotion Regulation, Mood, and Psychosis

Emotion regulation involves monitoring, evaluating, and modifying the type, timing, intensity, and expression of emotions (384) and is crucial to facilitating adaptive social functioning. Moreover, emotion regulation plays a critical role in disorders such as anxiety and depression, the latter representing more persistent states of mood disturbance (385). Inadequate emotion regulation in boys and men with KS is described in studies of executive function (315, 318, 328), as is related adverse coping behaviors and psychopathology (including emotional outbursts, avoidance, distraction seeking, passivity, and symptoms of anxiety and depression) (386).

Adults with KS struggle more to identify and verbalize their own emotions (372), and adults (379, 387, 388), as well as children (318, 335, 380), report feeling substantially more anxious and depressed. Clinically significant anxiety has an average reported prevalence of 20.5% among children with KS, while depressive disorders occur in an average of 26.9% of children and adults (331). Compared to the incidence in the general population of 4% for anxiety (World Health Organization, https://www.who.int/news-room/fact-sheets/detail/anxiety-disorders) and 5% for depression (https://www.who.int/news-room/fact-sheets/detail/depression), XXY represents a condition of increased vulnerability to psychopathology related to emotion and mood regulation.

Psychopathology related to more complex expressions of dysfunctional mood regulation or psychotic disorders in KS, such as bipolar disorder or schizophrenia, are in need of further investigation. In the largest-to-date study, bipolar disorder and psychosis occurred almost 4 times more often in a Swedish national sample of 860 patients with KS compared to matched population controls (n = 86 000) (334). In this line, small sample studies found symptoms related to schizophrenia to be significantly more pronounced in men with KS as compared to controls (372, 387, 389). Although several case-studies have provided insight into individual symptom presentations and treatments (390-393), large cross-sectional or registry-based studies are needed to accurately specify the nature and prevalence of bipolar and psychotic pathology in XXY. Also, the issue of biased sampling has been suggested to account for the increased incidence rates reported (394).

Psychosocial Factors and Quality of Life

In a large study, approximately 8 in 10 adolescents and adults with KS replied that their condition negatively affected their lives (395). Reviewing the literature, this statement makes immediate sense. In early reports of behaviors related to KS, inferior intellectual abilities, emotional immaturity and "character deviations" was suggested to account for the increased number of criminal records among men with KS (396-398), unfortunately nourishing negative stereotypes and misconceptions about the population. However, later and much more extensive studies found socioeconomic factors, not cognitive shortcomings, to largely explain the moderately increased risk of men with KS being convicted for crime (399). Individuals with KS experience a variety of adverse psychosocial and socioeconomic challenges. A longitudinal, register-based study including >1000 Danish men with KS and >100 000 controls has provided unique insights: 47, XXY men have lower educational and income levels, fewer partnerships and fatherhoods, and retire earlier (366). They have a harder time establishing a solid connection to the job market (133, 400, 401), and morbidity and mortality rates are significantly increased (121, 402). Moreover, many men with KS struggle to find qualified health care and counseling from professionals familiar with their condition (403, 404).

In ratings of QoL that measure experienced satisfaction with social life, health, life achievements, and self, individuals with KS further elaborate this picture. Significantly lower QoL is reported from peri-puberty into adulthood (361, 400, 403-406). In a large multicenter sample of men with KS, the lowest QoL was reported by men who experienced discrimination due to their condition, those who reported chronic mental or physical health problems, or those who participated the least in social activities (404). Phenotype severity and more pronounced physical manifestations of KS (eg, height, higher BMI, and smaller testes) were also negatively associated with QoL (361, 400). Peer relations and social support seem particularly important to increase QoL across age groups (328, 400), as does employment in adults (400). In other words, a sense of belonging and contributing may act as protective factors. To date, it has not been possible to establish a beneficial effect of T on QoL in either boys (361) or adults (407). Studying treatment effects in a longitudinal design, preferably with subjects blinded to treatment, is needed to fill this knowledge gap.

Current Evidence and Gaps in the Field of Cancer

Extensive register-based epidemiological studies from the United Kingdom, Denmark, and Sweden have shown that the general risk of cancer is not increased in men with KS (402, 408-410). However, it was shown that the incidence of specific cancers, such as breast cancer (121, 408, 409), mediastinal germ cell tumors (121, 409), as well as hematological malignancies including non-Hodgkin lymphoma and leukemia (410), is significantly increased. By contrast, the incidence of prostate cancer is significantly reduced (408, 410). Compared with the background population, mortality from lung cancer, breast cancer, and non-Hodgkin lymphoma was found to be increased, whereas mortality from prostate cancer was decreased in men with KS (408).

Male breast cancer constitutes less than 1% of all breast cancers and is regarded as a rare condition as opposed to among women, in whom the incidence of breast cancer ranges from 71 to 194 in 100 000 in European countries (411). Men with KS have a 20- to 50-fold increased risk of developing breast cancer compared to the general male population (412, 413), and it has been estimated that up to 7% of men with breast cancer have KS (412). However, due to the rarity of male breast cancer in general, only a few studies exist and the estimates in KS may be subject to selection bias. Future studies of the influence of risk factors, for example, gynecomastia and long-term TRT, are needed.

In the register-based study by Swerdlow et al, including 3518 men with KS (pure 47,XXY n = 3002, mosaic 47,XXY n = 320, other [comprising HGAs] n = 196), the incidence and mortality of breast cancer were significantly increased by 19.2 (95% CI 5.2 to 49.2) and 57.8 (95% CI 18.8 to 135.0) times, compared with the general male population. The largest mortality ratio in men was found in mosaic KS (408). However, when comparing mortality from breast cancer to the rates of women, the standardized mortality ratio was significantly lower in men with KS (SMR = 0.3, 95% CI 0.1-0.8) (408). The authors speculate that the increased risk of breast cancer could be related to increased serum concentrations of estradiol or, more likely, to the increased ratio between estradiol and T (408), although this has not been shown.

Mediastinal germ cell tumors are rare, and the reported risk of mediastinal germ cell tumors in KS varies from study to study, depending on the study design. In the study by Hasle et al, including 696 men with KS, 4 mediastinal germ cell tumors were observed, corresponding to a significantly increased relative risk of 66.7 (95% CI 17.9 to 170.7). The 4 patients were between 14 and 29 years old, and all tumors

were malignant non-seminomas (409). Bojesen et al reported 3 cases with mediastinal tumors in 832 men with KS compared to 1 among 4033 controls, with a hazard ratio of 14.2 (121). By contrast, no mediastinal tumors were observed in the Swerdlow et al study (408). Williams et al studied the reverse relationship in a cohort of 433 male patients aged 0 to 19 years with germ cell tumors (414). Thirteen (3%) of these boys turned out to have KS, corresponding to a relative risk of 18.8 (95% CI 11.7-30.0). Among the 13 patients with KS, 11 (84.6%) had extragonadal tumors, and 9 (81.8%) of the extragonadal tumors were mediastinal. For comparison, 16.8% of the non-KS patients had extragonadal tumors, and 31.8% were mediastinal. It was thus concluded that approximately one-third of the patients with mediastinal germ cell tumors had KS and that screening for KS may be warranted in pediatric and adolescent males with mediastinal germ cell tumors (414).

In a Swedish register-based study including 1085 men with KS, solid tumor risk significantly decreased with a standardized incidence ratio (SIR) of 0.66 (95% CI 0.47-0.90). By contrast, the general risk of hematological malignancy was significantly increased (SIR 2.72; 95% CI 1.61-4.30), with SIRs of 3.02 (95% CI 1.44-5.57) for non-Hodgkin lymphoma and 3.62 (95% CI 1.30-7.93) for leukemias (410). The authors speculate that genetic alterations associated with the extra X chromosome, such as X-linked immune-related genes and microRNAs, may be involved in the etiology of these cancers (410). However, the risk estimates in hematological cancers may be highly overestimated, since chromosome aberrations may be detected during cytogenetic evaluation of bone marrow that is routinely part of the evaluation of these conditions. Only a few epidemiological studies of cancer risk and mortality in KS exist, and these studies are limited by the low diagnosis rate of KS and, thereby, an inherent risk of selection bias. The results should, therefore, be interpreted with caution. However, based on the current evidence, clinical breast and axilla examination is suggested every second year in adults with KS (19). Screening for KS in pediatric and adolescent patients with mediastinal germ cell tumors may also be warranted (410).

Current Evidence and Gaps on Immune Function

Among the comorbidities associated with a KS diagnosis comes an increased prevalence of several autoimmune conditions, as evidenced, among others, by population and registry studies. Specifically, increased occurrences of Addison disease, type 1 diabetes mellitus, multiple sclerosis, autoimmune hypothyroidism, rheumatoid arthritis, Sjøgren syndrome and systemic lupus erythematosus (SLE), have all been described (121, 193, 198, 415, 416). Atopic conditions such as IgE-related food allergies and eosinophilic esophagitis have also been reported in various case series (417, 418).

In addition, KS seems to be characterized by both an organ-specific (anti-thyroglobulin and anti-thyroperoxidase, anti-insulin, GAD, IA-2, and ZnT8 antibodies) and a non-organ-specific (anti-nuclear antibodies, ANAs) humoral autoimmune tendency, possibly more so in subjects naïve to TRT (194, 198, 419, 420). Altered serum levels of both pro- and anti-inflammatory cytokines have also been reported by some authors in children and adults affected by KS, specifically IL-1α, IL-2, IL-4, IL-6, IL-10, IL-12 (421, 422), alongside higher total serum IgA, IgG, IgM concentrations and increased $CD3^+$, $CD4^+$, and $CD19^+$ cell counts, which showed a decrease after TRT start to healthy controls levels (422).

Although the causes of this predisposition in KS are only partially known, it has been suggested to originate from sex hormone differences and imbalance of the sex chromosomes, to resemble the disproportionate prevalence of autoimmune diseases in biological female vs male individuals, as well as in female individuals with 45,X Turner syndrome (423, 424). X-linked genes involved in immune response and escaping X chromosome inactivation, such as TLR7, IL3RA, CD40L, FOXP3, etc., have been suggested to play a role (424-426). More recently, it has been shown how the long noncoding RNA (lncRNA) Xist, responsible for X chromosome inactivation in individuals carrying 2 (or more) X chromosomes (427), together with its ribonucleoproteins, represent an important driver of sex-based autoimmunity, and the expression of a nonsilencing Xist form in male mice induces a more severe autoimmune response and both T and B cell reprogramming to resemble a female phenotype (427, 428).

It is not currently clear to what extent these immune system alterations may further affect the outcomes of other comorbidities associated with KS, such as the natural history of type 2 diabetes mellitus and its complications, for example, in the context of leg ulceration and (delayed) wound healing (285, 429).

Current Evidence and Gaps on the Timing and Pros and Cons of TRT

A limited number of randomized controlled trials on the effects of TRT have been conducted, and no evidence-based guidelines exist on how early, and through which approach, to start treatment, outside what is known for male hypogonadism in general (430).

Only one randomized study of TRT in infancy exists (281). In that study, 20 infants (aged 6 to 15 weeks) were randomized to 3 months of treatment with testosterone cypionate 25 mg intramuscularly every 28 days (n = 10) or no treatment. At baseline, there was no difference in fat mass between the 2 groups, but at 5 months of age, untreated infants had a significantly higher fat mass (15%) than controls. In contrast, no difference in fat mass between treated infants and controls was found. In addition, the increase in total mass, fat-free mass, length, stretched penile length, and growth velocity was greater in the treated infants (281).

In a double-blind, randomized, placebo-controlled study by Davis et al, 84 prepubertal boys with KS aged 4 to 12 years were randomized to treatment with low-dose synthetic oral androgen (oxandrolone) for 24 months (284, 431, 432). Boys in the oxandrolone-treated group exhibited significant reductions in body fat, triglycerides, and HDL cholesterol, and improvements in areas of visual-motor performance, anxiety, depression, and social functioning. Still, no effect on cognition and attention was found (284, 432). Notably, the risk of early gonadarche, pubarche, and advanced bone age was increased in the oxandrolone-treated boys (431).

Randomized controlled trials are needed to elucidate the influence of TRT on pubertal development and spermatogenesis in patients with KS. The timing of TRT initiation also appears crucial regarding peak bone mass and strength attainment, which in the general male population occurs between the

ages of 19 to 33 years (433, 434). This window partly corresponds to crucial events in the natural history of KS, in which Leydig cell dysfunction becomes evident from Tanner stage 4 onward as an altered T/LH ratio, preceding the decline in T and cfT concentrations, which typically becomes apparent during transition age and adulthood (101).

As discussed previously, TRT is likely to be introduced to men with KS at some stage in their lifespan. In particular, T has multiple effects on the vasculature (435, 436), and hypogonadism is known to be a cardiovascular risk factor in aging men (437).

A general discussion on the pros and cons of TRT in male hypogonadism is outside the scope of the present review; however, it is worth mentioning the results of the largest and longest double-blind placebo-controlled randomized trial recently published in the *New England Journal of Medicine* (438). The TRAVERSE trial enrolled 5204 men aged between 45 and 80 years old, with 15 304 life-years of follow-up. Men with KS were not excluded from this trial, although to participate, the men had to have preexisting CVD (438). The data demonstrate that in hypogonadism, TRT can correct anemia (439), increase libido (269), and improve bone health (178), with a low risk of adverse events (440). No significant differences were found in the composite of major cardiovascular events (304). A recent systematic review and meta-analysis of 799 articles encompassing 1144 men with KS and 1284 healthy controls showed that men with KS who did not receive TRT had impaired metabolic profiles (hyperglycemia and increased cholesterol), body composition (higher BMI and waist circumference) and bone health (reduced BMD) compared to age-matched controls (177). Of note, TRT improved body composition and BMD but not metabolic biochemistry. The combined evidence from the TRAVERSE and the TTrials (267) clearly showed that TRT in adult men with hypogonadism is safe. Whether TRT can have beneficial effects in reversing cardiometabolic comorbidities, is likely related to the timing TRT is started, the etiology of hypogonadism, the duration and the levels of T achieved during treatment (430, 441).

Based on our current understanding and the phenotypic variability of KS, it appears plausible to conceive that: (i) some outcomes of the syndrome are genetically (pre)determined (eg, testicular failure); (ii) others derive from the decline in T levels (eg, reduced libido) and could be reversed by prompt replacement therapy; (iii) some others might originate from the combined effect of (epi)genetic factors and lack of sufficient T in critical developmental programming windows such as fetal life and mini-puberty (eg, low bone health, increased adiposity and CVD risk); and (iv) a combination of maladaptive changes (eg, altered thyroid feedback, impaired vitamin D metabolism, etc.) determining the full spectrum of KS clinical phenotype.

Understanding the specific contribution of each of these factors to the various comorbidities characterizing the natural history of KS will allow us to understand which outcomes are modifiable/preventable and which are not. It is, therefore, clear that a significant research gap lies in understanding the ideal timing of TRT commencement, as well as T target values, and in the efficacy of other (pharmacological, behavioral, etc.) combined interventions to overcome comorbidities. Most experts agree that a timely TRT can improve several, but not all, unmet needs of patients affected by KS. Combination therapies, for example, with vitamin D, should be seriously considered.

Patient Engagement

Several patient support organizations currently exist for boys and men with KS and their families, including but not limited to the Association for X and Y Variations (genetic.org), the Klinefelter Syndrome Association (ksa-uk.net), the American Association for Klinefelter Syndrome Information and Support (aaksis.org) and the Living with XXY group (livingwithxxy.org). Private patient groups on social media platforms have also been reported as being beneficial for patient advocacy, education, and increasing feelings of empowerment regarding their condition (442). Guidelines regarding the care of children with conditions like KS recommend working in partnership with these groups (443). Support groups have been demonstrated to be effective in terms of encouraging individuals with KS to participate in research studies (444). In 2017, a European Union COST Action from the DSDnet group facilitated a workshop for 33 individuals with disorders/differences of sex development (DSD), their parents and representation from advisory groups and healthcare professionals to discuss key areas, including diagnosis, childhood, transition to adult services, and potential research topics, concluding that a life course approach should be a focus of future work (445). An international survey of healthcare professionals from 28 countries involved in caring for people with DSD rated research priorities to address the gaps in current knowledge, with QoL ranking highest (446). Any studies in the field of KS should seek the opinion of patients, patient advisory groups, and their carers regarding the research areas of highest priority to those affected by the condition.

The Value of Models in KS

As discussed briefly above, animal models of KS have proven invaluable in advancing our knowledge regarding the mechanisms of comorbidities associated with the condition, and a number of different models exist. The 41,XX^{Y} model is currently the most widely described mouse model, obtained by breeding males with a mutated Y chromosome (Y*) from a B6Ei.Lt-Y* strain with wild-type female mice (447). Using a 4-generation breeding scheme involving males with the structurally rearranged Y chromosome, XXY mice can be bred, resulting in 50% of live-born male offspring in the fourth generation having an XXY karyotype and a phenotype of small testes, decreased plasma T, increased plasma FSH, and evidence of neurocognitive delay (448). This model was first described in 1991 by Eicher and colleagues (449) and has since been comprehensively described. The mice demonstrate Leydig cell hyperactivation and germ cell loss, as seen in human males with KS, as well as similarities in bone metabolism and lack of androgenization, as reviewed recently by Wistuba and colleagues (450). This model does not, however, result in the same alterations in body proportions or the cardiovascular phenotype seen in men. Alternatively, XXY male mice can be bred by mating a wild-type female to a sex chromosome variant male (XYY) that could produce XY-containing sperm, resulting in a spermatogenesis phenotype with the arrest at the pro spermatogonial stage (220). Again, this model is best placed for assessing fertility instead of other aspects of KS in humans.

Cellular models also exist for the investigation of pathology in KS and other sex chromosome aneuploidies. Patient-

specific induced pluripotent stem cells (iPSCs) have been developed from individuals with KS by transfecting patient and control fibroblasts with nonmodified RNAs leading to cellular reprogramming and the development of condition-specific cell lines (451, 452). Development of these cellular lines can be challenging, particularly as 47,XXY-derived lines have been shown to have increased rates of cell death at germ cell differentiation compared to 46,XY cell lines (453). With appropriate technical expertise, however, such cell lines may obviate the need for animal models, provided the cells demonstrate that they are able to sustain their original phenotype with each cellular passage.

Standardized Data Collection

Natural History Disease Registries

Several epidemiological and outcome-based cohort studies have been performed using linked data sets from existing sources of routinely collected healthcare data (285, 305, 454). In recent years, rare disease registries have been developed to collect real-world data on specific conditions for promoting research and improvement in quality of care (455). In particular, the International Disorders of Sex Development (I-DSD) Registry, as part of the International Registries for Rare Conditions Affecting Sex Development and Maturation (https://sdmregistries.org) collates routinely collected data on boys and men with KS. Local country-specific registries include the Korean Endocrine Society Registry (288) and PEDSnet (https://pedsnet.org). Currently, none of these registries gather a core dataset which is unique to KS and given the clinical heterogeneity of KS, future work should focus on the development of a specific core outcome set (COS) for KS. A COS is a minimum consensus-based set of outcomes that should be reported in all clinical trials for a specific health condition or intervention to foster research homogeneity and minimize the risk of selective outcome reporting bias in studies (456). This COS could be the basis of forming a new international registry for KS, which would provide the opportunity to pool data from across the globe to gain a comprehensive understanding of the life course of the syndrome and provide evidence concerning outcomes and treatments that would otherwise be unattainable. An international priority setting partnership to develop age-specific COS is being developed for use in a proposed module for KS within the Sdmregistries.org platform, based on the currently reported outcomes in the medical literature for boys and men with KS. Additional data could be collated within an ethical and governance framework that would be expected of a high-quality registry (457). Patient-reported outcome measures (PROMs) could also be incorporated to ensure that the patient's voice is at the forefront of any ongoing work in the field of KS. This is particularly important in the face of recent evidence demonstrating that men with KS in the dsd-LIFE study scored lower for QoL scores on physical, social, and psychological domains (404). Overall, this would potentially accelerate the development of novel treatments to improve QoL in men with KS.

Suggested Research Priorities

While it is clear that there have been significant advances in our understanding of KS over recent years, many gaps persist. Table 1 highlights the key research priorities identified in our review. Some of the research gaps are currently being addressed by ongoing studies on KS, as summarized in Table 2

Table 1. Proposed research priorities for KS

Research area	Suggested research gap	Main refs.
Etiology and phenotypic variability	Methods to increase rates of diagnosis	(20, 23, 26, 32, 33, 42)
	Genetic predisposition to sex chromosome nondisjunction	(23, 26, 27, 32, 38, 42, 45, 46)
	Genomics and transcriptomics in people with KS	(76-78, 80-82)
Puberty	Timing of puberty and optimal timing for testosterone supplementation	(93, 95, 96, 101, 104, 106)
Fertility	Pathophysiology of microvascular alterations in the testes	(114-117)
	Temporal events of testicular cell degeneration	(83, 90, 96, 101, 108)
	Optimal timing of (m)TESE and sperm cryopreservation	(229-231, 239)
	Predictive factors for successful TESE	(101, 231, 239)
	Success of assisted reproduction techniques	(223, 225, 226, 228-230)
Bone	Prevention of reduced BMD (and fractures)	(123, 125, 128, 145, 159)
	Efficacy of TRT on bone health	(131, 176-178)
	Role of the bone-testicular axis	(146, 147, 159)
Thyroid	Clinical implications of altered HPT axis	(121, 192, 195, 198)
	Efficacy of levothyroxine therapy	(198)
Cardiometabolic	Mechanisms underlying increased CV risk	(285, 289, 296, 299-301)
	Mechanisms underlying VTE risk	(285, 305, 307, 308)
	Interventions to prevent/manage metabolic syndrome	(95, 120, 282, 284, 285, 293)
	Effects of TRT on cardiovascular health	(170, 177, 304)
Neurodevelopment	Differential effects of TRT, psychopharmacology, and therapeutic interventions on cognition and psychopathology	(311, 312, 317, 377, 383)
Quality of life	Patient-reported outcome measures (PROMs)	(404-407)
Research standardization	Core outcome set development	(455-457)

Abbreviations: BMD, bone mineral density; CV, cardiovascular; EF, executive function; HPT, hypothalamic-pituitary-thyroid; KS, Klinefelter syndrome; PROMs: patient-reported outcomes; (m)TESE: microscopic testicular sperm extraction; TRT, testosterone replacement therapy; VTE, venous thromboembolism.

Table 2. Active trials that include KS from the NIH and EMA databases

Study ID	Brief study title	Condition(s)	Study type	Phase	Enrollment	Main study outcome
NCT06294990 2023-505854-16-00	Klinefelter syndrome—the effect of Testosterone treatment In PubertY A randomized, double-blind placebo-controlled intervention study "The TiPY study"	• KS	Interventional: • testosterone	4	32	Changes in body composition: • Body fat % • Android/gynoid ratio • Muscle mass
NCT05498090	Interrogating Fatty Acid Metabolism Impairment and Clinical Correlates in Males With Klinefelter Syndrome	• KS	Interventional: • fenofibrate	4	44	• Maximal rate of fat oxidation • Skeletal muscle fat oxidation
NCT05586802	Sex Steroids Balance for Metabolic and Reproductive Health in Klinefelter Syndrome (KLIN-HEALTH)	• KS	Interventional: • anastrozole • hCG • testosterone • semaglutide	3	150	• Sperm retrieval rate at mTESE • Change in HOMA-IR
NCT05997706	Unraveling the Klinefelter's Disease Physiopathology (KLINEFELTER)	• KS	Interventional: • (m)TESE	NA	20	Cell counts, cells characterization by IF and cell-specific RTqPCR, cell karyotyping
NCT03836300	Parent-Infant Inter(X)Action Intervention (PIXI)	• KS and other neurogenetic disorders with associated developmental delays or intellectual and developmental disabilities	Interventional: • Behavioral (PIXI)	NA	120	• Social validity and acceptability (family satisfaction with aspects of the intervention) • Fidelity (family retention rate)
NCT04252001	Growing up With the Young Endocrine Support System (YESS!) (YESS)	• KS • TS • CAH and AD • HH • GHD • CPHD • AIS • Thyroid dysgenesis	Interventional: • YESS! game	NA	160	• Self-management and Transition to Adulthood with Rx (STARx) questionnaire
NCT04803474	Turner And Klinefelter Treatment Target Study (TAKTT)	• KS • TS	Observational	NA	370	• Relationship between hair T concentrations and QoL (EQ-5D-5L)
NCT03396562	The eXtroardinarY Babies Study: Natural History of Health and Neurodevelopment in Infants and Young Children With Sex Chromosome Trisomy	• KS • 47,XYY syndrome • Triple X syndrome • HGA with male and female phenotype	Observational	NA	300	Longitudinal descriptive statistics of: • Cognitive scores • Motor scores • Language scores
NCT05425953	Endocrine, Metabolic, Cardiovascular and Immunological Aspects of Sex Chromosome Abnormalities in Relation to Genotype (EMKISCA)	• KS • TS • 47,XYY syndrome • Triple X syndrome	Observational	NA	60	Epigenetic changes related to phenotype from blood, fat, muscle, skin, buccal swabs, and urine samples
NCT04463316	GROWing Up With Rare GENEtic Syndromes (GROW UR GENES)	• KS • Other rare syndromes or rare congenital diseases	Observational	NA	600	• Presence of physical health problems • Biochemistry and endocrine panel • Physical and psychological complaints • Medication use
NCT03809026	The Potential of Sperm Retrieved by Micro-TESE to Fertilize Vitrified/ Warmed Oocytes	• Men referred for mTESE for KS, spermatogenetic arrest, or failed previous sperm retrieval	Observational	NA	100	• Cleavage rate • Pregnancy rate • Fertilization rate

(*continued*)

Table 2. Continued

Study ID	Brief study title	Condition(s)	Study type	Phase	Enrollment	Main study outcome
NCT06373861	Generating Advancements Through Longitudinal Analysis in X and Y Variations (GALAXY)	• KS • 47,XYY syndrome • Triple X syndrome • HGA with male and female phenotype	Observational	NA	5000	Average number of chronic diagnoses per person: • Mental health (depression, anxiety, mood disorder NOS, psychotic disorder, ADHD, autistic disorder) • Cardiometabolic (obesity, dyslipidemia, hypertension) • Autoimmune(DM, hypo- and hyperthyroidism)

Active trials listed in clinicaltrials.gov and euclinicaltrials.eu databases.
Abbreviations: AD, Addison disease; ADHD, attention-deficit/hyperactivity disorder; AIS, androgen insensitivity syndrome; CAH, congenital adrenal hyperplasia; CPHD, combined pituitary hormone deficit; DM, diabetes mellitus; GHD, growth hormone deficiency; hCG, human chorionic gonadotropin; HGA, high-grade aneuploidies; HH, hypogonadotropic hypogonadism; HOMA-IR, homeostasis model of insulin resistance; IF, immunofluorescence; KS, Klinefelter syndrome; NOS, not otherwise specified; RTqPCR, real time quantitative polymerase chain reaction; mTESE, microscopic testicular sperm extraction; TS, Turner syndrome.

(derived from the clinicaltrials.gov and euclinicaltrials.eu databases). Of course, these are not exhaustive, and many other research areas are clinically significant. Decisions regarding research priorities should also be led by those affected by the condition, whether as individuals or caregivers. Overall, there is a requirement for prospective and longitudinal studies of people with KS to determine their changing healthcare needs as they progress through their lifespan. Randomized controlled trials are very demanding, and funding is scarce, but well-performed observational and registry studies can substitute for what is otherwise not feasible in rare disorders. These studies would be best initiated with international collaboration, to ensure that the data produced are valid to people with this condition across the world.

Conclusions

KS is a complex condition associated with a multitude of different comorbidities. That said, boys and men with KS lead meaningful and happy lives, which would be facilitated by increased knowledge regarding the condition. We demonstrate that the new horizons in this field are exciting and promise to change some of the current trajectories of morbidity seen in KS.

Acknowledgments

This work is generated within the European Reference Network for rare endocrine conditions (Endo-ERN). The authors would like to acknowledge all of the boys, the respective parents, and the men with KS who they see in their clinical practice, alongside those who provided written informed consent for their images to be taken and used for scientific publication.

Funding

A.K.L.H. is supported by an NES/CSO Clinical Lectureship PCL/21/05. L.A. is supported by Rigshospitalet's Research Council. I.D.C. is supported by the Novo Nordisk Foundation (NNF22OC0080944). F.C. is supported by the Italian Ministry of University and Research (MUR) through PRIN 2020 (CUP B89J22002140001). This work was supported by the European Union–NextGeneration EU through the Italian Ministry of University and Research (PNRR-M4C2-I1.3 Project PE_00000019 "HEAL ITALIA" to A.M.I., CUP B53C22004000006) and by internal funding to Sapienza University of Rome. The funder/sponsor did not participate in the work.

Author Contributions

A.K.L.H., S.F.A., and A.I. conceptualized the work. All authors contributed to writing the first draft, reviewing, and editing the final version.

Disclosures

The authors have nothing to disclose related to the content of the present work.

References

1. Zhao Y, Gardner EJ, Tuke MA, *et al.* Detection and characterization of male sex chromosome abnormalities in the UK Biobank study. *Genet Med*. 2022;24(9):1909-19.
2. Klinefelter HF Jr, Reifenstein EC Jr, Albright F Jr. Syndrome characterized by gynecomastia, aspermatogenesis without A-Leydigism, and increased excretion of follicle-stimulating hormone. *J Clin Endocrinol Metab*. 1942;2(11):615-27.
3. Bojesen A, Juul S, Gravholt CH. Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. *J Clin Endocrinol Metab*. 2003;88(2):622-6.
4. Berglund A, Viuff MH, Skakkebæk A, Chang S, Stochholm K, Gravholt CH. Changes in the cohort composition of turner syndrome and severe non-diagnosis of Klinefelter, 47,XXX and 47, XYY syndrome: a nationwide cohort study. *Orphanet J Rare Dis*. 2019;14(1):16.
5. Fraccaro M, Kaijser K, Lindsten J. A child with 49 chromosomes. *Lancet*. 1960;2(7156):899-902.
6. Bray P, Sister AJ. An XXXYY sex-chromosome anomaly. Report of a mentally deficient male. *JAMA*. 1963;184(3):179-82.
7. Salamanca-Gòmez F, Cortès R, Sànchez J, Armendares S. A 49, XXXYY male. *Am J Med Genet*. 1981;10(4):351-5.
8. Vorsanova SG, Demidova IA, Kolotii AD, *et al.* Klinefelter syndrome mosaicism in boys with neurodevelopmental disorders: a cohort study and an extension of the hypothesis. *Mol Cytogenet*. 2022;15(1):8.
9. Kleczkowska A, Fryns JP, Van den Berghe H. X-chromosome polysomy in the male. The Leuven experience 1966–1987. *Hum Genet*. 1988;80(1):16-22.

10. Gianfrilli D, Carlomagno F, Sciarra F, Lombardo F. *Different Karyotypes, Same Disease? Klinefelter's Syndrome*. Springer International Publishing; 2020:15-23.
11. Tartaglia N, Borodyanskaya M, Hall DA. Tremor in 48,XXYY syndrome. *Mov Disord*. 2009;24(13):2001-7.
12. Peet J, Weaver DD, Vance GH. 49,XXXXY: a distinct phenotype. Three new cases and review. *J Med Genet*. 1998;35(5):420-4.
13. Tartaglia N, Davis S, Hench A, *et al.* A new look at XXYY syndrome: medical and psychological features. *Am J Med Genet A*. 2008;146A(12):1509-22.
14. Visootsak J, Rosner B, Dykens E, Tartaglia N, Graham JM Jr. Behavioral phenotype of sex chromosome aneuploidies: 48, XXYY, 48,XXXY, and 49,XXXXY. *Am J Med Genet A*. 2007;143A(11):1198-203.
15. Spaziani M, Carlomagno F, Tarantino C, *et al.* From Klinefelter syndrome to high grade aneuploidies: expanding the gene-dosage effect of supernumerary X chromosomes. *J Clin Endocrinol Metab*. 2024;109(8):e1564-e1573.
16. Spaziani M, Mileno B, Rossi F, *et al.* Endocrine and metabolic evaluation of classic Klinefelter syndrome and high-grade aneuploidies of sexual chromosomes with male phenotype: are they different clinical conditions? *Eur J Endocrinol*. 2018;178(4): 343-52.
17. Ford CE, Jones KW, Miller OJ, *et al.* The chromosomes in a patient showing both mongolism and the Klinefelter syndrome. *Lancet*. 1959;273(7075):709-10.
18. Leisti JT, Aula PP, Hjelt LH. Two cases of (prepubertal) Klinefelter's syndrome with XXYY sex chromosomes. *Ann Hum Genet*. 1964;28(1-3):71-6.
19. Zitzmann M, Aksglaede L, Corona G, *et al.* European academy of andrology guidelines on Klinefelter Syndrome Endorsing Organization: European Society of Endocrinology. *Andrology*. 2021;9(1):145-67.
20. Thomas NS, Hassold TJ. Aberrant recombination and the origin of Klinefelter syndrome. *Hum Reprod Update*. 2003;9(4):309-17.
21. Jacobs PA, Hassold TJ, Whittington E, *et al.* Klinefelter's syndrome: an analysis of the origin of the additional sex chromosome using molecular probes. *Ann Hum Genet*. 1988;52(2):93-109.
22. Hassold T, Hunt PA, Sherman S. Trisomy in humans: incidence, origin and etiology. *Curr Opin Genet Dev*. 1993;3(3):398-403.
23. Hassold TJ, Sherman SL, Pettay D, Page DC, Jacobs PA. XY chromosome nondisjunction in man is associated with diminished recombination in the pseudoautosomal region. *Am J Hum Genet*. 1991;49(2):253-60.
24. Hassold TJ. Nondisjunction in the human male. *Curr Top Dev Biol*. 1998;37:383-406.
25. Thomas NS, Collins AR, Hassold TJ, Jacobs PA. A reinvestigation of non-disjunction resulting in 47, XXY males of paternal origin. *Eur J Hum Genet*. 2000;8(10):805-8.
26. Arnedo N, Templado C, Sánchez-Blanque Y, Rajmil O, Nogués C. Sperm aneuploidy in fathers of Klinefelter's syndrome offspring assessed by multicolour fluorescent in situ hybridization using probes for chromosomes 6, 13, 18, 21, 22, X and Y. *Hum Reprod*. 2006;21(2):524-8.
27. Sun F, Mikhaail-Philips M, Oliver-Bonet M, *et al.* Reduced meiotic recombination on the XY bivalent is correlated with an increased incidence of sex chromosome aneuploidy in men with non-obstructive azoospermia. *Mol Hum Reprod*. 2008;14(7): 399-404.
28. Lorda-Sanchez I, Binkert F, Maechler M, Robinson WP, Schinzel AA. Reduced recombination and paternal age effect in Klinefelter syndrome. *Hum Genet*. 1992;89(5):524-30.
29. Bonomi M, Rochira V, Pasquali D, *et al.* Klinefelter syndrome (KS): genetics, clinical phenotype and hypogonadism. *J Endocrinol Invest*. 2017;40(2):123-34.
30. De Souza E, Morris JK; EUROCAT Working Group. Case-control analysis of paternal age and trisomic anomalies. *Arch Dis Child*. 2010;95(11):893-7.
31. Uroz L, Templado C. Meiotic non-disjunction mechanisms in human fertile males. *Hum Reprod*. 2012;27(5):1518-24.
32. Eskenazi B, Wyrobek AJ, Kidd SA, *et al.* Sperm aneuploidy in fathers of children with paternally and maternally inherited Klinefelter syndrome. *Hum Reprod*. 2002;17(3):576-83.
33. Ferlin A, Garolla A, Foresta C. Chromosome abnormalities in sperm of individuals with constitutional sex chromosomal abnormalities. *Cytogenet Genome Res*. 2005;111(3-4):310-6.
34. Selice R, Di Mambro A, Garolla A, *et al.* Spermatogenesis in Klinefelter syndrome. *J Endocrinol Invest*. 2010;33(11):789-93.
35. Smyth CM, Bremner WJ. Klinefelter syndrome. *Arch Intern Med*. 1998;158(12):1309-14.
36. Vialard F, Bailly M, Bouazzi H, *et al.* The high frequency of sperm aneuploidy in Klinefelter patients and in nonobstructive azoospermia is due to meiotic errors in euploid spermatocytes. *J Androl*. 2012;33(6):1352-9.
37. La Volpe A, Barchi M. Meiotic double strand breaks repair in sexually reproducing eukaryotes: we are not all equal. *Exp Cell Res*. 2012;318(12):1333-9.
38. Lane S, Kauppi L. Meiotic spindle assembly checkpoint and aneuploidy in males versus females. *Cell Mol Life Sci*. 2019;76(6): 1135-50.
39. Gray S, Cohen PE. Control of meiotic crossovers: from double-strand break formation to designation. *Annu Rev Genet*. 2016;50(1):175-210.
40. Cole F, Kauppi L, Lange J, *et al.* Homeostatic control of recombination is implemented progressively in mouse meiosis. *Nat Cell Biol*. 2012;14(4):424-30.
41. Shi Q, Spriggs E, Field LL, Ko E, Barclay L, Martin RH. Single sperm typing demonstrates that reduced recombination is associated with the production of aneuploid 24,XY human sperm. *Am J Med Genet*. 2001;99(1):34-8.
42. Giannattasio T, Testa E, Faieta M, *et al.* The proper interplay between the expression of Spo11 splice isoforms and the structure of the pseudoautosomal region promotes XY chromosomes recombination. *Cell Mol Life Sci*. 2023;80(10):279.
43. Acquaviva L, Boekhout M, Karasu ME, *et al.* Ensuring meiotic DNA break formation in the mouse pseudoautosomal region. *Nature*. 2020;582(7812):426-31.
44. Sciarra F, Pelloni M, Faja F, *et al.* Incidence of Y chromosome microdeletions in patients with Klinefelter syndrome. *J Endocrinol Invest*. 2019;42(7):833-42.
45. Liu C, Liu H, Zhang H, *et al.* Paternal USP26 mutations raise Klinefelter syndrome risk in the offspring of mice and humans. *EMBO J*. 2021;40(13):e106864.
46. Fitz-James MH, Cavalli G. Molecular mechanisms of transgenerational epigenetic inheritance. *Nat Rev Genet*. 2022;23(6):325-41.
47. Boškovič A, Rando OJ. Transgenerational epigenetic inheritance. *Annu Rev Genet*. 2018;52(1):21-41.
48. Peeters SB, Posynick BJ, Brown CJ. Out of the silence: insights into how genes escape X-chromosome inactivation. *Epigenomes*. 2023;7(4):29.
49. Ottesen AM, Aksglaede L, Garn I, *et al.* Increased number of sex chromosomes affects height in a nonlinear fashion: a study of 305 patients with sex chromosome aneuploidy. *Am J Med Genet A*. 2010;152A(5):1206-12.
50. Imamura M. Role of loss and skew of X chromosome inactivation for developing myelodysplastic syndrome in patients with Klinefelter syndrome: a brief review of the literature. *Leuk Res Rep*. 2023;20:100396.
51. Astro V, Fiacco E, Cardona-Londoño KJ, *et al.* A transcriptomic signature of X chromosome overdosage in Saudi Klinefelter syndrome induced pluripotent stem cells. *Endocr Connect*. 2023;12(5):e220515.
52. Astro V, Alowaysi M, Fiacco E, *et al.* Pseudoautosomal region 1 overdosage affects the global transcriptome in iPSCs from patients with Klinefelter syndrome and high-grade X chromosome aneuploidies. *Front Cell Dev Biol*. 2021;9:801597.

53. Tüttelmann F, Gromoll J. Novel genetic aspects of Klinefelter's syndrome. *Mol Hum Reprod*. 2010;16(6):386-95.
54. Mehta A, Malek-Jones M, Bolyakov A, Mielnik A, Schlegel PN, Paduch DA. Methylation-specific PCR allows for fast diagnosis of X chromosome disomy and reveals skewed inactivation of the X chromosome in men with Klinefelter syndrome. *J Androl*. 2012;33(5):955-62.
55. Simonetti L, Ferreira LGA, Vidi AC, *et al.* Intelligence quotient variability in Klinefelter syndrome is associated with GTPBP6 expression under regulation of X-chromosome inactivation pattern. *Front Genet*. 2021;12:724625.
56. Chang S, Skakkebæk A, Trolle C, *et al.* Anthropometry in Klinefelter syndrome—multifactorial influences due to CAG length, testosterone treatment and possibly intrauterine hypogonadism. *J Clin Endocrinol Metab*. 2015;100(3):E508-E517.
57. Skakkebaek A, Bojesen A, Kristensen MK, *et al.* Neuropsychology and brain morphology in Klinefelter syndrome—the impact of genetics. *Andrology*. 2014;2(4):632-640.
58. Bojesen A, Hertz JM, Gravholt CH. Genotype and phenotype in Klinefelter syndrome—impact of androgen receptor polymorphism and skewed X inactivation. *Int J Androl*. 2011;34(6pt2):e642-e648.
59. Ross JL, Roeltgen DP, Stefanatos G, *et al.* Cognitive and motor development during childhood in boys with Klinefelter syndrome. *Am J Med Genet A*. 2008;146A(6):708-719.
60. Zinn AR, Ramos P, Elder FF, Kowal K, Samango-Sprouse C, Ross JL. Androgen receptor CAGn repeat length influences phenotype of 47,XXY (Klinefelter) syndrome. *J Clin Endocrinol Metab*. 2005;90(9):5041-5046.
61. Zeger MPD, Zinn AR, Lahlou N, *et al.* Effect of ascertainment and genetic features on the phenotype of Klinefelter syndrome. *J Pediatr*. 2008;152(5):716-722.
62. Stemkens D, Roza T, Verrij L, *et al.* Is there an influence of X-chromosomal imprinting on the phenotype in Klinefelter syndrome? A clinical and molecular genetic study of 61 cases. *Clin Genet*. 2006;70(1):43-48.
63. Bruining H, van Rijn S, Swaab H, *et al.* The parent-of-origin of the extra X chromosome may differentially affect psychopathology in Klinefelter syndrome. *Biol Psychiatry*. 2010;68(12):1156-1162.
64. Wikström AM, Painter JN, Raivio T, Aittomäki K, Dunkel L. Genetic features of the X chromosome affect pubertal development and testicular degeneration in adolescent boys with Klinefelter syndrome. *Clin Endocrinol*. 2006;65(1):92-97.
65. Zitzmann M. The role of the CAG repeat androgen receptor polymorphism in andrology. *Front Horm Res*. 2009;37:52-61.
66. Rocha RO, Billerbeck AEC, Pinto EM, *et al.* The degree of external genitalia virilization in girls with 21-hydroxylase deficiency appears to be influenced by the CAG repeats in the androgen receptor gene. *Clin Endocrinol*. 2008;68(2):226-232.
67. Dejager S, Bry-Gauillard H, Bruckert E, *et al.* A comprehensive endocrine description of Kennedy's disease revealing androgen insensitivity linked to CAG repeat length. *J Clin Endocrinol Metab*. 2002;87(8):3893-3901.
68. Zitzmann M, Depenbusch M, Gromoll J, Nieschlag E. X-chromosome inactivation patterns and androgen receptor functionality influence phenotype and social characteristics as well as pharmacogenetics of testosterone therapy in Klinefelter patients. *J Clin Endocrinol Metab*. 2004;89(12):6208-6217.
69. Rohayem J, Nieschlag E, Zitzmann M, Kliesch S. Testicular function during puberty and young adulthood in patients with Klinefelter's syndrome with and without spermatozoa in seminal fluid. *Andrology*. 2016;4(6):1178-1186.
70. Foresta C, Caretta N, Palego P, *et al.* Reduced artery diameters in Klinefelter syndrome. *Int J Androl*. 2012;35(5):720-725.
71. Ferlin A, Schipilliti M, Vinanzi C, *et al.* Bone mass in subjects with Klinefelter syndrome: role of testosterone levels and androgen receptor gene CAG polymorphism. *J Clin Endocrinol Metab*. 2011;96(4):E739-E745.
72. Lanfranco F, Kamischke A, Zitzmann M, Nieschlag E. Klinefelter's syndrome. *Lancet*. 2004;364(9430):273-283.
73. Gravholt CH, Chang S, Wallentin M, Fedder J, Moore P, Skakkebæk A. Klinefelter syndrome: integrating genetics, neuropsychology, and endocrinology. *Endocr Rev*. 2018;39(4):389-423.
74. Samplaski MK, Lo KC, Grober ED, Millar A, Dimitromanolakis A, Jarvi KA. Phenotypic differences in mosaic Klinefelter patients as compared with non-mosaic Klinefelter patients. *Fertil Steril*. 2014;101(4):950-955.
75. Garcia-Quevedo L, Blanco J, Sarrate Z, Català V, Bassas L, Vidal F. Hidden mosaicism in patients with Klinefelter's syndrome: implications for genetic reproductive counselling. *Hum Reprod*. 2011;26(12):3486-3493.
76. Skakkebæk A, Nielsen MM, Trolle C, *et al.* DNA hypermethylation and differential gene expression associated with Klinefelter syndrome. *Sci Rep*. 2018;8(1):13740.
77. Wan ES, Qiu W, Morrow J, *et al.* Genome-wide site-specific differential methylation in the blood of individuals with Klinefelter syndrome. *Mol Reprod Dev*. 2015;82(5):377-386.
78. Huang J, Zhang L, Deng H, Chang L, Liu Q, Liu P. Global transcriptome analysis of peripheral blood identifies the most significantly down-regulated genes associated with metabolism regulation in Klinefelter syndrome. *Mol Reprod Dev*. 2015;82(1):17-25.
79. Sharma A, Jamil MA, Nuesgen N, *et al.* DNA methylation signature in peripheral blood reveals distinct characteristics of human X chromosome numerical aberrations. *Clin Epigenetics*. 2015;7(1):76.
80. Belling K, Russo F, Jensen AB, *et al.* Klinefelter syndrome comorbidities linked to increased X chromosome gene dosage and altered protein interactome activity. *Hum Mol Genet*. 2017;26(7):1219-1229.
81. Tallaksen HBL, Johannsen EB, Just J, Viuff MH, Gravholt CH, Skakkebæk A. The multi-omic landscape of sex chromosome abnormalities: current status and future directions. *Endocr Connect*. 2023;12(9):e230011.
82. Johannsen EB, Just J, Viuff MH, *et al.* Sex chromosome aneuploidies give rise to changes in the circular RNA profile: a circular transcriptome-wide study of Turner and Klinefelter syndrome across different tissues. *Front Genet*. 2022;13:928874.
83. Aksglaede L, Wikström AM, Rajpert-De Meyts E, Dunkel L, Skakkebaek NE, Juul A. Natural history of seminiferous tubule degeneration in Klinefelter syndrome. *Hum Reprod Update*. 2006;12(1):39-48.
84. Winge SB, Dalgaard MD, Jensen JM, *et al.* Transcriptome profiling of fetal Klinefelter testis tissue reveals a possible involvement of long non-coding RNAs in gonocyte maturation. *Hum Mol Genet*. 2018;27(3):430-439.
85. Wikström AM, Raivio T, Hadziselimovic F, Wikström S, Tuuri T, Dunkel L. Klinefelter syndrome in adolescence: onset of puberty is associated with accelerated germ cell depletion. *J Clin Endocrinol Metab*. 2004;89(5):2263-2270.
86. Busch AS, Ljubicic ML, Upners EN, *et al.* Dynamic changes of reproductive hormones in male minipuberty: temporal dissociation of Leydig and Sertoli cell activity. *J Clin Endocrinol Metab*. 2022;107(6):1560-1568.
87. Scheutz Henriksen L, Holm Petersen J, Skakkebæk NE, *et al.* Serum testosterone levels in 3-month-old boys predict their semen quality as young adults. *J Clin Endocrinol Metab*. 2022;107(7):1965-1975.
88. Lahlou N, Fennoy I, Carel J-C, Roger M. Inhibin B and anti-Müllerian hormone, but not testosterone levels, are normal in infants with nonmosaic Klinefelter syndrome. *J Clin Endocrinol Metab*. 2004;89(4):1864-1868.
89. Ross JL, Samango-Sprouse C, Lahlou N, Kowal K, Elder FF, Zinn A. Early androgen deficiency in infants and young boys with 47, XXY Klinefelter syndrome. *Horm Res*. 2005;64(1):39-45.

90. Cabrol S, Ross JL, Fennoy I, Bouvattier C, Roger M, Lahlou N. Assessment of Leydig and Sertoli cell functions in infants with nonmosaic Klinefelter syndrome: insulin-like peptide 3 levels are normal and positively correlated with LH levels. *J Clin Endocrinol Metab*. 2011;96(4):E746-E753.
91. Lahlou N, Fennoy I, Ross JL, Bouvattier C, Roger M. Clinical and hormonal status of infants with nonmosaic XXY karyotype. *Acta Paediatr*. 2011;100(6):824-829.
92. Aksglaede L, Petersen JH, Main KM, Skakkebaek NE, Juul A. High normal testosterone levels in infants with non-mosaic Klinefelter's syndrome. *Eur J Endocrinol*. 2007;157(3):345-350.
93. Spaziani M, Granato S, Liberati N, *et al*. From mini-puberty to pre-puberty: early impairment of the hypothalamus-pituitary-gonadal axis with normal testicular function in children with non-mosaic Klinefelter syndrome. *J Endocrinol Invest*. 2021;44(1):127-138.
94. Aksglaede L, Davis S, Ross JL, Juul A. Congenital causes of hypergonadotropic hypogonadism: anorchia and Klinefelter syndrome. In: Foresta C, Gianfrilli D, eds. *Pediatric and Adolescent Andrology. Trends in Andrology and Sexual Medicine*. Springer; 2021:127-145.
95. Davis S, Lahlou N, Bardsley M, *et al*. Gonadal function is associated with cardiometabolic health in pre-pubertal boys with Klinefelter syndrome. *Andrology*. 2016;4(6):1169-1177.
96. Aksglaede L, Christiansen P, Sørensen K, *et al*. Serum concentrations of Anti-Müllerian Hormone (AMH) in 95 patients with Klinefelter syndrome with or without cryptorchidism. *Acta Paediatr*. 2011;100(6):839-845.
97. Grinspon RP, Rey RA. Anti-Müllerian hormone and sertoli cell function in paediatric male hypogonadism. *Horm Res Paediatr*. 2010;73(2):81-92.
98. Lee MM, Donahoe PK, Hasegawa T, *et al*. Mullerian inhibiting substance in humans: normal levels from infancy to adulthood. *J Clin Endocrinol Metab*. 1996;81(2):571-576.
99. Grinspon RP, Loreti N, Braslavsky D, *et al*. Sertoli cell markers in the diagnosis of paediatric male hypogonadism. *J Pediatr Endocrinol Metab*. 2012;25(1-2):3-11.
100. Rosenfeld RG, Nicodemus BC. The transition from adolescence to adult life: physiology of the 'transition' phase and its evolutionary basis. *Horm Res*. 2003;60(Suppl 1):74-77.
101. Pozza C, Sesti F, Tenuta M, *et al*. Testicular dysfunction in 47, XXY boys: when it all begins. A semilongitudinal study. *J Clin Endocrinol Metab*. 2023;108(10):2486-2499.
102. Sørensen K, Aksglaede L, Petersen JH, Juul A. Recent changes in pubertal timing in healthy Danish boys: associations with body mass index. *J Clin Endocrinol Metab*. 2010;95(1):263-270.
103. Christiansen P, Andersson AM, Skakkebaek NE. Longitudinal studies of inhibin B levels in boys and young adults with Klinefelter syndrome. *J Clin Endocrinol Metab*. 2003;88(2):888-891.
104. Aksglaede L, Skakkebaek NE, Almstrup K, Juul A. Clinical and biological parameters in 166 boys, adolescents and adults with nonmosaic Klinefelter syndrome: a Copenhagen experience. *Acta Paediatr*. 2011;100(6):793-806.
105. Van Saen D, Vloeberghs V, Gies I, *et al*. When does germ cell loss and fibrosis occur in patients with Klinefelter syndrome? *Hum Reprod*. 2018;33(6):1009-1022.
106. Tanner M, Miettinen PJ, Hero M, Toppari J, Raivio T. Onset and progression of puberty in Klinefelter syndrome. *Clin Endocrinol*. 2022;96(3):363-370.
107. Bastida MG, Rey RA, Bergadá I, *et al*. Establishment of testicular endocrine function impairment during childhood and puberty in boys with Klinefelter syndrome. *Clin Endocrinol*. 2007;67(6):863-870.
108. Pozza C, Pofi R, Tenuta M, *et al*. Clinical presentation, management and follow-up of 83 patients with Leydig cell tumors of the testis: a prospective case-cohort study. *Hum Reprod*. 2019;34(8):1389-1403.
109. Holm M, Rajpert-De Meyts E, Andersson A-M, Skakkebaek NE. Leydig cell micronodules are a common finding in testicular biopsies from men with impaired spermatogenesis and are associated with decreased testosterone/LH ratio. *J Pathol*. 2003;199(3):378-386.
110. Aksglaede L, Andersson A-M, Jørgensen N, *et al*. Primary testicular failure in Klinefelter's syndrome: the use of bivariate luteinizing hormone-testosterone reference charts. *Clin Endocrinol*. 2007;66(2):276-281.
111. Giannetta E, Gianfrilli D, Barbagallo F, Isidori AM, Lenzi A. Subclinical male hypogonadism. *Best Pract Res Clin Endocrinol Metab*. 2012;26(4):539-550.
112. Carto C, Loloi J, Campbell K, Ramasamy R. Testosterone therapy in men with Klinefelter syndrome: analysis of a global federated research network. *Androg Clin Res Ther*. 2022;3(1):208-212.
113. Pozzi E, Boeri L, Capogrosso P, *et al*. Rates of hypogonadism forms in Klinefelter patients undergoing testicular sperm extraction: a multicenter cross-sectional study. *Andrology*. 2020;8(6):1705-1711.
114. Tuttelmann F, Damm OS, Luetjens CM, *et al*. Intratesticular testosterone is increased in men with Klinefelter syndrome and may not be released into the bloodstream owing to altered testicular vascularization– a preliminary report. *Andrology*. 2014;2(2):275-281.
115. Wistuba J, Beumer C, Warmeling A-S, *et al*. Testicular blood supply is altered in the 41,XX* Klinefelter syndrome mouse model. *Sci Rep*. 2020;10(1):14369.
116. Carlomagno F, Pozza C, Tenuta M, *et al*. Testicular microvascular flow is altered in Klinefelter syndrome and predicts circulating testosterone. *J Clin Endocrinol Metab*. 2022;107(1):e236-e245.
117. Johannsen EB, Skakkebæk A, Kalucka JM, Fedder J, Gravholt CH, Just J. The testicular microvasculature in Klinefelter syndrome is immature with compromised integrity and characterized by excessive inflammatory cross-talk. *Hum Reprod*. 2023;38(12):2339-2349.
118. Ferlin A, Selice R, Carraro U, Foresta C. Testicular function and bone metabolism--beyond testosterone. *Nat Rev Endocrinol*. 2013;9(9):548-554.
119. Callewaert F, Sinnesael M, Gielen E, Boonen S, Vanderschueren D. Skeletal sexual dimorphism: relative contribution of sex steroids, GH-IGF1, and mechanical loading. *J Endocrinol*. 2010;207(2):127-134.
120. Swerdlow AJ, Higgins CD, Schoemaker MJ, Wright AF, Jacobs PA; United Kingdom Clinical Cytogenetics Group. Mortality in patients with Klinefelter syndrome in Britain: a cohort study. *J Clin Endocrinol Metab*. 2005;90(12):6516-6522.
121. Bojesen A, Juul S, Birkebaek NH, Gravholt CH. Morbidity in Klinefelter syndrome: a Danish register study based on hospital discharge diagnoses. *J Clin Endocrinol Metab*. 2006;91(4):1254-1260.
122. Vena W, Pizzocaro A, Indirli R, *et al*. Prevalence and determinants of radiological vertebral fractures in patients with Klinefelter syndrome. *Andrology*. 2020;8(6):1699-1704.
123. Bojesen A, Birkebæk N, Kristensen K, *et al*. Bone mineral density in Klinefelter syndrome is reduced and primarily determined by muscle strength and resorptive markers, but not directly by testosterone. *Osteoporos Int*. 2011;22(5):1441-1450.
124. van den Bergh JP, Hermus AR, Spruyt AI, Sweep CG, Corstens FH, Smals AG. Bone mineral density and quantitative ultrasound parameters in patients with Klinefelter's syndrome after long-term testosterone substitution. *Osteoporos Int*. 2001;12(1):55-62.
125. Vogiatzi MG, Davis SM, Ross JL. Cortical bone mass is low in boys with Klinefelter syndrome and improves with oxandrolone. *J Endocr Soc*. 2021;5(4):bvab016.
126. Aksglaede L, Molgaard C, Skakkebaek NE, Juul A. Normal bone mineral content but unfavourable muscle/fat ratio in Klinefelter syndrome. *Arch Dis Child*. 2008;93(1):30-34.

127. Stagi S, Di Tommaso M, Manoni C, *et al.* Bone mineral status in children and adolescents with Klinefelter syndrome. *Int J Endocrinol*. 2016;2016:3032759.
128. López Krabbe HV, Holm Petersen J, Asserhøj LL, *et al.* Reproductive hormones, bone mineral content, body composition, and testosterone therapy in boys and adolescents with Klinefelter syndrome. *Endocr Connect*. 2023;12(7):e230031.
129. Vena W, Carrone F, Delbarba A, *et al.* Body composition, trabecular bone score and vertebral fractures in subjects with Klinefelter syndrome. *J Endocrinol Invest*. 2023;46(2):297-304.
130. Shanbhogue VV, Hansen S, Jørgensen NR, Brixen K, Gravholt CH. Bone geometry, volumetric density, microarchitecture, and estimated bone strength assessed by HR-pQCT in Klinefelter syndrome. *J Bone Miner Res*. 2014;29(11):2474-2482.
131. Piot A, Plotton I, Boutroy S, *et al.* Klinefelter bone microarchitecture evolution with testosterone replacement therapy. *Calcif Tissue Int*. 2022;111(1):35-46.
132. Ferlin A, Selice R, Di Mambro A, *et al.* Role of vitamin D levels and vitamin D supplementation on bone mineral density in Klinefelter syndrome. *Osteoporos Int*. 2015;26(8):2193-2202.
133. Skakkebæk A, Moore PJ, Chang S, Fedder J, Gravholt CH. Quality of life in men with Klinefelter syndrome: the impact of genotype, health, socioeconomics, and sexual function. *Genet Med*. 2018;20(2):214-222.
134. Spiekermann J, Sinningen K, Hanusch B, *et al.* Cardiorespiratory fitness in adolescents and young adults with Klinefelter syndrome—a pilot study. *Front Endocrinol*. 2023;14:1106118.
135. De Toni L, Agoulnik AI, Sandri M, Foresta C, Ferlin A. INSL3 in the muscolo-skeletal system. *Mol Cell Endocrinol*. 2019;487:12-17.
136. Foresta C, Bettella A, Vinanzi C, *et al.* A novel circulating hormone of testis origin in humans. *J Clin Endocrinol Metab*. 2004;89(12):5952-5958.
137. Anand-Ivell R, Wohlgemuth J, Haren MT, *et al.* Peripheral INSL3 concentrations decline with age in a large population of Australian men. *Int J Androl*. 2006;29(6):618-626.
138. Facondo P, Delbarba A, Maffezzoni F, Cappelli C, Ferlin A. INSL3: a marker of Leydig cell function and testis-bone-skeletal muscle network. *Protein Pept Lett*. 2020;27(12):1246-1252.
139. Grande G, Graziani A, Di Mambro A, Selice R, Ferlin A. Osteoporosis and bone metabolism in patients with Klinefelter syndrome. *Endocr Connect*. 2023;12(8):e230058.
140. Wikström AM, Bay K, Hero M, Andersson A-M, Dunkel L. Serum insulin-like factor 3 levels during puberty in healthy boys and boys with Klinefelter syndrome. *J Clin Endocrinol Metab*. 2006;91(11):4705-4708.
141. Bay K, Hartung S, Ivell R, *et al.* Insulin-like factor 3 serum levels in 135 normal men and 85 men with testicular disorders: relationship to the luteinizing hormone-testosterone axis. *J Clin Endocrinol Metab*. 2005;90(6):3410-3418.
142. Johannsen TH, Ljubicic ML, Young J, *et al.* Serum insulin-like factor 3 quantification by LC-MS/MS in male patients with hypogonadotropic hypogonadism and Klinefelter syndrome. *Endocrine*. 2021;71(3):578-585.
143. Santi D, Ivell R, Anand-Ivell R, *et al.* Effects of acute hCG stimulation on serum INSL3 and 25-OH vitamin D in Klinefelter syndrome. *Andrology*. 2020;8(6):1720-1727.
144. Overvad S, Bay K, Bojesen A, Gravholt CH. Low INSL3 in Klinefelter syndrome is related to osteocalcin, testosterone treatment and body composition, as well as measures of the hypothalamic-pituitary-gonadal axis. *Andrology*. 2014;2(3):421-427.
145. Di Nisio A, De Toni L, Rocca MS, *et al.* Negative association between sclerostin and INSL3 in isolated human osteocytes and in Klinefelter syndrome: new hints for testis-bone crosstalk. *J Clin Endocrinol Metab*. 2018;103(5):2033-2041.
146. Oury F, Sumara G, Sumara O, *et al.* Endocrine regulation of male fertility by the skeleton. *Cell*. 2011;144(5):796-809.
147. Oury F, Ferron M, Huizhen W, *et al.* Osteocalcin regulates murine and human fertility through a pancreas-bone-testis axis. *J Clin Invest*. 2013;123(6):2421-2433.
148. Shan C, Yue J, Liu W. Broadening the role of osteocalcin in the hypothalamic-pituitary-gonadal axis. *J Endocrinol*. 2021;249(2):R43-R51.
149. Pinto DC, Delgado IC, Yang H, *et al.* Osteocalcin of maternal and embryonic origins synergize to establish homeostasis in offspring. *bioRxiv 552969*. https://doi.org/10.1101/2023.08.11.552969, 14 August 2023, preprint: not peer reviewed.
150. Khrimian L, Obri A, Ramos-Brossier M, *et al.* Gpr158 mediates osteocalcin's regulation of cognition. *J Exp Med*. 2017;214(10):2859-2873.
151. Guo X-Z, Shan C, Hou Y-F, *et al.* Osteocalcin ameliorates motor dysfunction in a 6-hydroxydopamine-induced Parkinson's disease rat model through AKT/GSK3β signaling. *Front Mol Neurosci*. 2018;11:343.
152. Glatigny M, Moriceau S, Rivagorda M, *et al.* Autophagy is required for memory formation and reverses age-related memory decline. *Curr Biol*. 2019;29(3):435-448.e8.
153. Shan C, Ghosh A, Guo X-Z, *et al.* Roles for osteocalcin in brain signalling: implications in cognition- and motor-related disorders. *Mol Brain*. 2019;12(1):23.
154. Liao M, Guo X, Yu X, *et al.* Role of metabolic factors in the association between osteocalcin and testosterone in Chinese men. *J Clin Endocrinol Metab*. 2013;98(8):3463-3469.
155. Kirmani S, Atkinson EJ, Melton LJ 3rd, Riggs BL, Amin S, Khosla S. Relationship of testosterone and osteocalcin levels during growth. *J Bone Miner Res*. 2011;26(9):2212-2216.
156. Hannemann A, Breer S, Wallaschofski H, *et al.* Osteocalcin is associated with testosterone in the general population and selected patients with bone disorders. *Andrology*. 2013;1(3):469-474.
157. Limonard EJ, van Schoor NM, de Jongh RT, Lips P, Fliers E, Bisschop PH. Osteocalcin and the pituitary-gonadal axis in older men: a population-based study. *Clin Endocrinol*. 2015;82(5):753-759.
158. Yeap BB, Alfonso H, Chubb SAP, *et al.* Higher serum undercarboxylated osteocalcin and other bone turnover markers are associated with reduced diabetes risk and lower estradiol concentrations in older men. *J Clin Endocrinol Metab*. 2015;100(1):63-71.
159. Carlomagno F, Hasenmajer V, Spaziani M, *et al.* Total osteocalcin levels are independently associated with worse testicular function and a higher degree of hypothalamic-pituitary-gonadal axis activation in Klinefelter syndrome. *J Endocrinol Invest*. 2024;47(12):3049-3056.
160. Spaziani M, Carlomagno F, Tenuta M, *et al.* Extra-gonadal and non-canonical effects of FSH in males. *Pharmaceuticals*. 2023;16(6):813.
161. Khosla S, Monroe DG. Regulation of bone metabolism by sex steroids. *Cold Spring Harb Perspect Med*. 2018;8(1):a031211.
162. Sowers MR, Zheng H, McConnell D, Nan B, Harlow S, Randolph JF Jr. Follicle stimulating hormone and its rate of change in defining menopause transition stages. *J Clin Endocrinol Metab*. 2008;93(10):3958-3964.
163. Robinson LJ, Tourkova I, Wang Y, *et al.* FSH-receptor isoforms and FSH-dependent gene transcription in human monocytes and osteoclasts. *Biochem Biophys Res Commun*. 2010;394(1):12-17.
164. Sun L, Peng Y, Sharrow AC, *et al.* FSH directly regulates bone mass. *Cell*. 2006;125(2):247-260.
165. Iqbal J, Sun L, Kumar TR, Blair HC, Zaidi M. Follicle-stimulating hormone stimulates TNF production from immune cells to enhance osteoblast and osteoclast formation. *Proc Natl Acad Sci U S A*. 2006;103(40):14925-14930.
166. Chin K-Y. The relationship between follicle-stimulating hormone and bone health: alternative explanation for bone loss beyond oestrogen? *Int J Med Sci*. 2018;15(12):1373-1383.

167. Giovanelli L, Quinton R, Cangiano B, *et al.* FSH and bone: comparison between males with central versus primary hypogonadism. *Front Endocrinol*. 2022;13:939897.
168. Karim N, MacDonald D, Dolan AL, Fogelman I, Wierzbicki AS, Hampson G. The relationship between gonadotrophins, gonadal hormones and bone mass in men. *Clin Endocrinol*. 2008;68(1): 94-101.
169. Antonio L, Priskorn L, Olesen IA, Petersen JH, Vanderschueren D, Jørgensen N. High serum FSH is not a risk factor for low bone mineral density in infertile men. *Bone*. 2020;136:115366.
170. Kabilan A, Skakkebæk A, Chang S, Gravholt CH. Evaluation of the efficacy of transdermal and injection testosterone therapy in Klinefelter syndrome: a real-life study. *J Endocr Soc*. 2021;5(6): bvab062.
171. Russell N, Grossmann M. MECHANISMS IN ENDOCRINOLOGY: estradiol as a male hormone. *Eur J Endocrinol*. 2019;181(1):R23-R43.
172. Santi D, De Vincentis S, Scaltriti S, Rochira V. Relative hyperestrogenism in Klinefelter syndrome: results from a meta-analysis. *Endocrine*. 2019;64(2):209-219.
173. Vanderschueren D, Laurent MR, Claessens F, *et al.* Sex steroid actions in male bone. *Endocr Rev*. 2014;35(6):906-960.
174. Vandenput L, Ohlsson C. Estrogens as regulators of bone health in men. *Nat Rev Endocrinol*. 2009;5(8):437-443.
175. Stepan JJ, Burckhardt P, Hána V. The effects of three-month intravenous ibandronate on bone mineral density and bone remodeling in Klinefelter's syndrome: the influence of vitamin D deficiency and hormonal status. *Bone*. 2003;33(4):589-596.
176. Tahani N, Nieddu L, Prossomariti G, *et al.* Long-term effect of testosterone replacement therapy on bone in hypogonadal men with Klinefelter syndrome. *Endocrine*. 2018;61(2):327-335.
177. Pizzocaro A, Vena W, Condorelli R, *et al.* Testosterone treatment in male patients with Klinefelter syndrome: a systematic review and meta-analysis. *J Endocrinol Invest*. 2020;43(12):1675-1687.
178. Snyder PJ, Bauer DC, Ellenberg SS, *et al.* Testosterone treatment and fractures in men with hypogonadism. *N Engl J Med*. 2024;390(3):203-211.
179. Grossmann M, Anawalt BD. Breaking news—testosterone treatment and fractures in older men. *N Engl J Med*. 2024;390(3): 267-268.
180. Bhasin S, Ellenberg SS, Storer TW, *et al.* Effect of testosterone replacement on measures of mobility in older men with mobility limitation and low testosterone concentrations: secondary analyses of the testosterone trials. *Lancet Diabetes Endocrinol*. 2018;6(11):879-890.
181. Davis TE, Canfield CJ, Herman RH, Goler D. Thyroid function in patients with aspermiogenesis and testicular tubular sclerosis. *N Engl J Med*. 1963;268(4):178-182.
182. Berthezene F, Pousset G, Rollet J, Darsy P, Riou JP. [Proceedings: thyrotropin deficiencies in patients with Klinefelter's syndrome (author's transl)]. *Ann Endocrinol*. 1974;35(5):583-584.
183. Ozawa Y, Shishiba Y. Lack of TRH-induced TSH secretion in a patient with Klinefelter's syndrome: a case report. *Endocrinol Jpn*. 1975;22(3):269-273.
184. Smals AG, Kloppenborg PW, Lequin RL, Beex L, Ross A, Benraad TJ. The pituitary-thyroid axis in Klinefelter's syndrome. *Acta Endocrinol (Copenh)*. 1977;84(1):72-79.
185. Cheikh IE, Hamilton BP, Hsu TH, Wiswell JG. Studies using releasing factors in Klinefelter's syndrome: the responses of LH and FSH to luteinizing hormone-releasing hormone and of prolactin and TSH to thyrotropin-releasing hormone before and after testosterone. *Psychoneuroendocrinology*. 1981;6(1):37-44.
186. Hughes TA, Borsey DQ. Partial hypopituitarism in Klinefelter's syndrome. *Br J Clin Pract*. 1995;49(3):162-163.
187. Portioli I, Visioli O. [Latent thyroid insufficiency in Klinefelter's syndrome]. *Folia Endocrinol*. 1968;21(1):40-47.
188. Wortsman J, Moses HW, Dufau ML. Increased incidence of thyroid disease among men with hypergonadotropic hypogonadism. *Am J Med*. 1986;80(6):1055-1059.
189. Hsueh WA, Hsu TH, Federman DD. Endocrine features of Klinefelter's syndrome. *Medicine (Baltimore)*. 1978;57(5): 447-461.
190. Yap PL, Renton WB, Price WH, Fyffe JA, Lidgard GP. Response to thyrotrophin releasing hormone in Klinefelter's syndrome. *Clin Endocrinol*. 1978;8(3):237-240.
191. Burman KD, Dimond RC, Noel GL, Earll JM, Frantz AG, Wartofsky L. Klinefelter's syndrome: examination of thyroid function, and the TSH and PRL responses to thyrotropin-releasing hormone prior to and after testosterone administration. *J Clin Endocrinol Metab*. 1975;41(6):1161-1166.
192. Bjørn A-MB, Bojesen A, Gravholt CH, Laurberg P. Hypothyroidism secondary to hypothalamic-pituitary dysfunction may be part of the phenotype in Klinefelter syndrome: a case-control study. *J Clin Endocrinol Metab*. 2009;94(7):2478-2481.
193. Seminog OO, Seminog AB, Yeates D, Goldacre MJ. Associations between Klinefelter's syndrome and autoimmune diseases: English national record linkage studies. *Autoimmunity*. 2015;48(2): 125-128.
194. Panimolle F, Tiberti C, Granato S, *et al.* Evidence of increased humoral endocrine organ-specific autoimmunity in severe and classic X-chromosome aneuploidies in comparison with 46,XY control subjects. *Autoimmunity*. 2018;51(4):175-182.
195. Balercia G, Bonomi M, Giagulli VA, *et al.* Thyroid function in Klinefelter syndrome: a multicentre study from KING group. *J Endocrinol Invest*. 2019;42(10):1199-1204.
196. Sciarra F, Campolo F, Franceschini E, Carlomagno F, Venneri MA. Gender-specific impact of sex hormones on the immune system. *Int J Mol Sci*. 2023;24(7):6302.
197. Tahani N, Ruga G, Granato S, *et al.* A combined form of hypothyroidism in pubertal patients with non-mosaic Klinefelter syndrome. *Endocrine*. 2017;55(2):513-518.
198. Carlomagno F, Minnetti M, Angelini F, *et al.* Altered thyroid feedback loop in Klinefelter syndrome: from infancy through the transition to adulthood. *J Clin Endocrinol Metab*. 2023;108(11): e1329-e1340.
199. Xu W-Q, Yuan Y, Chen Y, Luo T, Chen H-Y. Birth of a boy after intracytoplasmic sperm injection using ejaculated spermatozoa from a nonmosaic klinefelter syndrome man with normal sperm motility: a case report. *Front Genet*. 2022;13:989701.
200. Gurbuz AS, Salvarci A, Ozcimen N, Zamani AG. Early morphokinetic monitoring of embryos after intracytoplasmic sperm injection with fresh ejaculate sperm in nonmosaic Klinefelter syndrome: a different presentation. *Case Rep Genet*. 2015;2015: 827656.
201. Ni T-X, Yan J-H, Wang B, *et al.* Outcomes of 13 ICSI-PGD cycles with ejaculated spermatozoa in patients with Klinefelter syndrome. *Asian J Androl*. 2016;18(3):498-499.
202. Zhang C, Peng H, Hu Y. Twin pregnancy obtention of patient with nonmosaic Klinefelter's syndrome and his wife with chromosome 9 inversion by ICSI treatment. *Int J Fertil Steril*. 2013;7(2): 142-146.
203. Greco E, Scarselli F, Minasi MG, *et al.* Birth of 16 healthy children after ICSI in cases of nonmosaic Klinefelter syndrome. *Hum Reprod*. 2013;28(5):1155-1160.
204. Ichioka K, Utsunomiya N, Kohei N, Ueda N, Inoue K, Terai A. Adult onset of declining spermatogenesis in a man with nonmosaic Klinefelter's syndrome. *Fertil Steril*. 2006;85(5):1511.e1-1511.e2.
205. Komori S, Horiuchi I, Hamada Y, *et al.* Birth of healthy neonates after intracytoplasmic injection of ejaculated or testicular spermatozoa from men with nonmosaic Klinefelter's syndrome: a report of 2 cases. *J Reprod Med*. 2004;49(2):126-130.
206. Staessen C, Tournaye H, Van Assche E, *et al.* PGD in 47,XXY Klinefelter's syndrome patients. *Hum Reprod Update*. 2003;9(4):319-330.
207. Crüger D, Toft B, Agerholm I, Fedder J, Hald F, Bruun-Petersen G. Birth of a healthy girl after ICSI with ejaculated spermatozoa from a man with non-mosaic Klinefelter's syndrome. *Hum Reprod*. 2001;16(9):1909-1911.

208. Ron-El R, Strassburger D, Gelman-Kohan S, Friedler S, Raziel A, Appelman Z. A 47,XXY fetus conceived after ICSI of spermatozoa from a patient with non-mosaic Klinefelter's syndrome: case report. *Hum Reprod*. 2000;15(8):1804-1806.
209. Kitamura M, Matsumiya K, Koga M, *et al*. Ejaculated spermatozoa in patients with non-mosaic Klinefelter's syndrome. *Int J Urol*. 2000;7(3):88-92; discussion 93–4.
210. Bourne H, Stern K, Clarke G, Pertile M, Speirs A, Baker HW. Delivery of normal twins following the intracytoplasmic injection of spermatozoa from a patient with 47,XXY Klinefelter's syndrome. *Hum Reprod*. 1997;12(11):2447-2450.
211. Elzeiny H, Agresta F, Stevens J, Gardner DK. A rare case of mosaic Klinefelter syndrome in a 45-year-old man leading to successful live birth through ejaculated spermatozoa: a case report and literature review. *Front Genet*. 2023;14:1242180.
212. Yu Y, Xi Q, Jing J, *et al*. Intracytoplasmic sperm injection outcome of ejaculated spermatozoa from a man with mosaic Klinefelter's syndrome: case report and literature review. *J Int Med Res*. 2018;46(10):4323-4331.
213. Akashi T, Fuse H, Kojima Y, Hayashi M, Honda S. Birth after intracytoplasmic sperm injection of ejaculated spermatozoa from a man with mosaic Klinefelter's syndrome. *Asian J Androl*. 2005;7(2):217-220.
214. Bielanska M, Tan SL, Ao A. Fluorescence in-situ hybridization of sex chromosomes in spermatozoa and spare preimplantation embryos of a Klinefelter 46,XY/47,XXY male. *Hum Reprod*. 2000;15(2):440-444.
215. Harari O, Bourne H, Baker G, Gronow M, Johnston I. High fertilization rate with intracytoplasmic sperm injection in mosaic Klinefelter's syndrome. *Fertil Steril*. 1995;63(1):182-184.
216. Laron Z, Dickerman Z, Zamir R, Galatzer A. Paternity in Klinefelter's syndrome—a case report. *Arch Androl*. 1982;8(2):149-151.
217. Li DZ. Paternity in Klinefelter syndrome—another case report. *J Endocrinol Invest*. 2011;34(7):570.
218. Maiburg M, Repping S, Giltay J. The genetic origin of Klinefelter syndrome and its effect on spermatogenesis. *Fertil Steril*. 2012;98(2):253-260.
219. Sciurano RB, Luna Hisano CV, Rahn MI, *et al*. Focal spermatogenesis originates in euploid germ cells in classical Klinefelter patients. *Hum Reprod*. 2009;24(9):2353-2360.
220. Hirota T, Ohta H, Powell BE, *et al*. Fertile offspring from sterile sex chromosome trisomic mice. *Science*. 2017;357(6354):932-935.
221. Winge SB, Skakkebaek NE, Aksglaede L, *et al*. X-chromosome loss rescues Sertoli cell maturation and spermatogenesis in Klinefelter syndrome. *Cell Death Dis*. 2024;15(6):396.
222. Schlegel PN. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. *Hum Reprod*. 1999;14(1):131-135.
223. Gao S, Yang X, Xiao X, *et al*. Outcomes and affecting factors for ICSI and microTESE treatments in nonobstructive azoospermia patients with different etiologies: a retrospective analysis. *Front Endocrinol*. 2022;13:1006208.
224. Kocamanoglu F, Ayas B, Bolat MS, Abur U, Bolat R, Asci R. Endocrine, sexual and reproductive functions in patients with Klinefelter syndrome compared to non-obstructive azoospermic patients. *Int J Clin Pract*. 2021;75(8):e14294.
225. Corona G, Minhas S, Giwercman A, *et al*. Sperm recovery and ICSI outcomes in men with non-obstructive azoospermia: a systematic review and meta-analysis. *Hum Reprod Update*. 2019;25(6):733-757.
226. Zhang H-L, Zhao L-M, Mao J-M, *et al*. Sperm retrieval rates and clinical outcomes for patients with different causes of azoospermia who undergo microdissection testicular sperm extraction-intracytoplasmic sperm injection. *Asian J Androl*. 2021;23(1):59-63.
227. Okuyama N, Obata R, Oka N, *et al*. Long-term clinical outcomes of testicular sperm extraction and intracytoplasmic sperm injection for infertile men. *Reprod Med Biol*. 2018;17(1):82-88.
228. Sabbaghian M, Modarresi T, Hosseinifar H, *et al*. Comparison of sperm retrieval and intracytoplasmic sperm injection outcome in patients with and without Klinefelter syndrome. *Urology*. 2014;83(1):107-110.
229. Corona G, Pizzocaro A, Lanfranco F, *et al*. Sperm recovery and ICSI outcomes in Klinefelter syndrome: a systematic review and meta-analysis. *Hum Reprod Update*. 2017;23(3):265-275.
230. Boeri L, Palmisano F, Preto M, *et al*. Sperm retrieval rates in nonmosaic Klinefelter patients undergoing testicular sperm extraction: what expectations do we have in the real-life setting? *Andrology*. 2020;8(3):680-687.
231. Renault L, Labrune E, Giscard d'Estaing S, *et al*. Delaying testicular sperm extraction in 47,XXY Klinefelter patients does not impair the sperm retrieval rate, and AMH levels are higher when TESE is positive. *Hum Reprod*. 2022;37(11):2518-2531.
232. Vernaeve V, Staessen C, Verheyen G, Van Steirteghem A, Devroey P, Tournaye H. Can biological or clinical parameters predict testicular sperm recovery in 47,XXY Klinefelter's syndrome patients? *Hum Reprod*. 2004;19(5):1135-1139.
233. Okada H, Goda K, Yamamoto Y, *et al*. Age as a limiting factor for successful sperm retrieval in patients with nonmosaic Klinefelter's syndrome. *Fertil Steril*. 2005;84(6):1662-1664.
234. Emre Bakircioglu M, Erden HF, Kaplancan T, Ciray N, Bener F, Bahceci M. Aging may adversely affect testicular sperm recovery in patients with Klinefelter syndrome. *Urology*. 2006;68(5):1082-1086.
235. Bakircioglu ME, Ulug U, Erden HF, *et al*. Klinefelter syndrome: does it confer a bad prognosis in treatment of nonobstructive azoospermia? *Fertil Steril*. 2011;95(5):1696-1699.
236. Ferhi K, Avakian R, Griveau JF, Guille F. Age as only predictive factor for successful sperm recovery in patients with Klinefelter's syndrome. *Andrologia*. 2009;41(2):84-87.
237. Ramasamy R, Ricci JA, Palermo GD, Gosden LV, Rosenwaks Z, Schlegel PN. Successful fertility treatment for Klinefelter's syndrome. *J Urol*. 2009;182(3):1108-1113.
238. Özkan B, Coşkuner ER, Güdelci T. Predictive factors and intracytoplasmic sperm injection results for sperm retrieval by microdissection Testicular Sperm Extraction (micro-TESE) in patients with Klinefelter syndrome. *Urology*. 2022;161:59-64.
239. Kang C, Punjani N, Kashanian JA, Schlegel PN. Age, sperm retrieval, and testicular histology in Klinefelter syndrome. *J Urol*. 2024;211(1):163-169.
240. Hawksworth DJ, Szafran AA, Jordan PW, Dobs AS, Herati AS. Infertility in patients with Klinefelter syndrome: optimal timing for sperm and testicular tissue cryopreservation. *Rev Urol*. 2018;20(2):56-62.
241. Franik S, Hoeijmakers Y, D'Hauwers K, *et al*. Klinefelter syndrome and fertility: sperm preservation should not be offered to children with Klinefelter syndrome. *Hum Reprod*. 2016;31(9):1952-1959.
242. Gassei K, Orwig KE. Experimental methods to preserve male fertility and treat male factor infertility. *Fertil Steril*. 2016;105(2):256-266.
243. Ibtisham F, Honaramooz A. Spermatogonial stem cells for in vitro spermatogenesis and in vivo restoration of fertility. *Cells*. 2020;9(3):745.
244. Pook CJ, Cocca A, Grandone A, Al-Hussini M, Lam W. The evidence for fertility preservation in pediatric Klinefelter syndrome. *Front Reprod Health*. 2021;3:629179.
245. Corona G, Petrone L, Paggi F, *et al*. Sexual dysfunction in subjects with Klinefelter's syndrome. *Int J Androl*. 2010;33(4):574-580.
246. Yoshida A, Miura K, Nagao K, Hara H, Ishii N, Shirai M. Sexual function and clinical features of patients with Klinefelter's syndrome with the chief complaint of male infertility. *Int J Androl*. 1997;20(2):80-85.
247. El Bardisi H, Majzoub A, Al Said S, Alnawasra H, Dabbous Z, Arafa M. Sexual dysfunction in Klinefelter's syndrome patients. *Andrologia*. 2017;49(6):e12670.

248. Rosen RC, Fisher WA, Eardley I, *et al.* The multinational Men's Attitudes to Life Events and Sexuality (MALES) study: I. Prevalence of erectile dysfunction and related health concerns in the general population. *Curr Med Res Opin*. 2004;20(5):607-617.
249. Pavlidi P, Kokras N, Dalla C. Antidepressants' effects on testosterone and estrogens: what do we know? *Eur J Pharmacol*. 2021;899:173998.
250. Bella AJ, Shamloul R. Psychotropics and sexual dysfunction. *Cent European J Urol*. 2014;66(4):466-471.
251. Serretti A, Chiesa A. Treatment-emergent sexual dysfunction related to antidepressants: a meta-analysis. *J Clin Psychopharmacol*. 2009;29(3):259-266.
252. Montejo AL, Majadas S, Rico-Villademoros F, *et al.* Frequency of sexual dysfunction in patients with a psychotic disorder receiving antipsychotics. *J Sex Med*. 2010;7(10):3404-3413.
253. Trinchieri M, Trinchieri M, Perletti G, *et al.* Erectile and ejaculatory dysfunction associated with use of psychotropic drugs: a systematic review. *J Sex Med*. 2021;18(8):1354-1363.
254. Bala A, Nguyen HMT, Hellstrom WJG. Post-SSRI sexual dysfunction: a literature review. *Sex Med Rev*. 2018;6(1):29-34.
255. Hirschfeld RMA. Long-term side effects of SSRIs: sexual dysfunction and weight gain. *J Clin Psychiatry*. 2003;64 Suppl 18:20-24.
256. Safer DJ. Raising the minimum effective dose of serotonin reuptake inhibitor antidepressants: adverse drug events. *J Clin Psychopharmacol*. 2016;36(5):483-491.
257. Higgins A, Nash M, Lynch AM. Antidepressant-associated sexual dysfunction: impact, effects, and treatment. *Drug Healthc Patient Saf*. 2010;2:141-150.
258. Allen K, Baban A, Munjiza J, Pappa S. Management of antipsychotic-related sexual dysfunction: systematic review. *J Sex Med*. 2019;16(12):1978-1987.
259. Montejo AL, de Alarcón R, Prieto N, Acosta JM, Buch B, Montejo L. Management strategies for antipsychotic-related sexual dysfunction: a clinical approach. *J Clin Med Res*. 2021;10(2):308.
260. Bobes J, Garc A-Portilla MP, Rejas J, *et al.* Frequency of sexual dysfunction and other reproductive side-effects in patients with schizophrenia treated with risperidone, olanzapine, quetiapine, or haloperidol: the results of the EIRE study. *J Sex Marital Ther*. 2003;29(2):125-147.
261. Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, McKinlay JB. Impotence and its medical and psychosocial correlates: results of the Massachusetts male aging study. *J Urol*. 1994;151(1):54-61.
262. Ferlin A, Selice R, Angelini S, *et al.* Endocrine and psychological aspects of sexual dysfunction in Klinefelter patients. *Andrology*. 2018;6(3):414-419.
263. De Rocco Ponce M, Selice R, Di Mambro A, De Toni L, Foresta C, Garolla A. Estradiol-testosterone imbalance is associated with erectile dysfunction in patients with Klinefelter syndrome. *J Clin Med Res*. 2021;10(11):2319.
264. Aversa A, Isidori AM, Greco EA, *et al.* Hormonal supplementation and erectile dysfunction. *Eur Urol*. 2004;45(5):535-538.
265. Skakkebaek NE, Bancroft J, Davidson DW, Warner P. Androgen replacement with oral testosterone undecanoate in hypogonadal men: a double blind controlled study. *Clin Endocrinol*. 1981;14(1):49-61.
266. Wang C, Swerdloff RS, Iranmanesh A, *et al.* Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. *J Clin Endocrinol Metab*. 2000;85(8):2839-2853.
267. Snyder PJ, Bhasin S, Cunningham GR, *et al.* Effects of testosterone treatment in older men. *N Engl J Med*. 2016;374(7):611-624.
268. Cunningham GR, Stephens-Shields AJ, Rosen RC, *et al.* Testosterone treatment and sexual function in older men with low testosterone levels. *J Clin Endocrinol Metab*. 2016;101(8):3096-3104.
269. Pencina KM, Travison TG, Cunningham GR, *et al.* Effect of testosterone replacement therapy on sexual function and hypogonadal symptoms in men with hypogonadism. *J Clin Endocrinol Metab*. 2024;109(2):569-580.
270. Bhasin S, Seidman S, Travison TG, *et al.* Depressive syndromes in men with hypogonadism in the TRAVERSE trial: response to testosterone replacement therapy. *J Clin Endocrinol Metab*. 2024;109(7):1814-1826.
271. Wu FC, Bancroft J, Davidson DW, Nicol K. The behavioural effects of testosterone undecanoate in adult men with Klinefelter's syndrome: a controlled study. *Clin Endocrinol*. 1982;16(5):489-497.
272. Fisher AD, Castellini G, Casale H, *et al.* Hypersexuality, paraphilic behaviors, and gender dysphoria in individuals with Klinefelter's syndrome. *J Sex Med*. 2015;12(12):2413-2424.
273. Davies GW, Parkinson J. Gender dysphoria in Klinefelter's syndrome: three cases. *Australas Psychiatry*. 2018;26(3):313-314.
274. Fernández R, Guillamón A, Gómez-Gil E, *et al.* Analyses of karyotype by G-banding and high-resolution microarrays in a gender dysphoria population. *Genes Genomics*. 2018;40(5):465-473.
275. Maillefer A, Sabe M, Coste C, Bartolomei J, Jaafar J, Sentissi O. Sexual identity disorder and psychosis in Klinefelter syndrome: a synthesis of literature and a case report. *J Nerv Ment Dis*. 2019;207(2):121-125.
276. Harkin C, Elander J. A qualitative exploration of experiences of gender identity and gender questioning among adults with Klinefelter syndrome/XXY. *J Genet Couns*. Published online July 22, 2024. Doi: 10.1002/jgc4.1952.
277. Kreukels BPC, Köhler B, Nordenström A, *et al.* Gender dysphoria and gender change in disorders of sex development/intersex conditions: results from the dsd-LIFE study. *J Sex Med*. 2018;15(5):777-785.
278. Liang B, Cheung AS, Nolan BJ. Clinical features and prevalence of Klinefelter syndrome in transgender individuals: a systematic review. *Clin Endocrinol*. 2022;97(1):3-12.
279. van de Grift TC, Cohen-Kettenis PT, de Vries ALC, Kreukels BPC. Body image and self-esteem in disorders of sex development: a European multicenter study. *Health Psychol*. 2018;37(4):334-343.
280. Butler G. Incidence of gynaecomastia in Klinefelter syndrome adolescents and outcome of testosterone treatment. *Eur J Pediatr*. 2021;180(10):3201-3207.
281. Davis SM, Reynolds RM, Dabelea DM, Zeitler PS, Tartaglia NR. Testosterone treatment in infants with 47,XXY: effects on body composition. *J Endocr Soc*. 2019;3(12):2276-2285.
282. Bardsley MZ, Falkner B, Kowal K, Ross JL. Insulin resistance and metabolic syndrome in prepubertal boys with Klinefelter syndrome. *Acta Paediatr*. 2011;100(6):866-870.
283. Davis SM, DeKlotz S, Nadeau KJ, Kelsey MM, Zeitler PS, Tartaglia NR. High prevalence of cardiometabolic risk features in adolescents with 47,XXY/Klinefelter syndrome. *Am J Med Genet C Semin Med Genet*. 2020;184(2):327-333.
284. Davis SM, Cox-Martin MG, Bardsley MZ, Kowal K, Zeitler PS, Ross JL. Effects of oxandrolone on cardiometabolic health in boys with Klinefelter syndrome: a randomized controlled trial. *J Clin Endocrinol Metab*. 2017;102(1):176-184.
285. Bojesen A, Kristensen K, Birkebaek NH, *et al.* The metabolic syndrome is frequent in Klinefelter's syndrome and is associated with abdominal obesity and hypogonadism. *Diabetes Care*. 2006;29(7):1591-1598.
286. Bojesen A, Gravholt CH. Morbidity and mortality in Klinefelter syndrome (47,XXY). *Acta Paediatr*. 2011;100(6):807-813.
287. Ishikawa T, Yamaguchi K, Kondo Y, Takenaka A, Fujisawa M. Metabolic syndrome in men with Klinefelter's syndrome. *Urology*. 2008;71(6):1109-1113.
288. Han SJ, Kim K-S, Kim W, *et al.* Obesity and hyperglycemia in Korean men with Klinefelter syndrome: the Korean endocrine society registry. *Endocrinol Metab (Seoul)*. 2016;31(4):598-603.
289. Demirci I, Haymana C, Candemir B, *et al.* Triglyceride-glucose index levels in patients with Klinefelter syndrome and its relationship with endothelial dysfunction and insulin resistance: a

cross-sectional observational study. *Arch Endocrinol Metab*. 2023;67(3):378-384.
290. Davis SM, Nokoff NJ, Furniss A, *et al.* Population-based assessment of cardiometabolic-related diagnoses in youth with Klinefelter syndrome: a PEDSnet study. *J Clin Endocrinol Metab*. 2022;107(5):e1850-e1859.
291. Salzano A, D'Assante R, Heaney LM, *et al.* Klinefelter syndrome, insulin resistance, metabolic syndrome, and diabetes: review of literature and clinical perspectives. *Endocrine*. 2018;61(2):194-203.
292. Panimolle F, Tiberti C, Granato S, *et al.* Screening of endocrine organ-specific humoral autoimmunity in 47, XXY Klinefelter's syndrome reveals a significant increase in diabetes-specific immunoreactivity in comparison with healthy control men. *Endocrine*. 2016;52(1):157-164.
293. Giagulli VA, Carbone MD, Ramunni MI, *et al.* Adding liraglutide to lifestyle changes, metformin and testosterone therapy boosts erectile function in diabetic obese men with overt hypogonadism. *Andrology*. 2015;3(6):1094-1103.
294. Rosenthal A. Cardiovascular malformations in Klinefelter's syndrome: report of three cases. *J Pediatr*. 1972;80(3):471-473.
295. Bianchini E, Guala A, Golemati S, *et al.* The ultrasound window into vascular ageing: a technology review by the VascAgeNet COST action. *J Ultrasound Med*. 2023;42(10):2183-2213.
296. Andersen NH, Bojesen A, Kristensen K, *et al.* Left ventricular dysfunction in Klinefelter syndrome is associated to insulin resistance, abdominal adiposity and hypogonadism. *Clin Endocrinol*. 2008;69(5):785-791.
297. Borlaug BA, Paulus WJ. Heart failure with preserved ejection fraction: pathophysiology, diagnosis, and treatment. *Eur Heart J*. 2011;32(6):670-679.
298. Pasquali D, Arcopinto M, Renzullo A, *et al.* Cardiovascular abnormalities in Klinefelter syndrome. *Int J Cardiol*. 2013;168(2):754-759.
299. Haymana C, Aydogdu A, Demirci I, *et al.* Increased endothelial dysfunction and insulin resistance in patients with Klinefelter syndrome. *Endocr Metab Immune Disord Drug Targets*. 2018;18(4):401-406.
300. Di Mambro A, Ferlin A, De Toni L, Selice R, Caretta N, Foresta C. Endothelial progenitor cells as a new cardiovascular risk factor in Klinefelter's syndrome. *Mol Hum Reprod*. 2010;16(6):411-417.
301. Granato S, Barbaro G, Di Giorgio MR, *et al.* Epicardial fat: the role of testosterone and lipid metabolism in a cohort of patients with Klinefelter syndrome. *Metabolism*. 2019;95:21-26.
302. Soisson V, Brailly-Tabard S, Helmer C, *et al.* A J-shaped association between plasma testosterone and risk of ischemic arterial event in elderly men: the French 3C cohort study. *Maturitas*. 2013;75(3):282-288.
303. Sesti F, Pofi R, Pozza C, Minnetti M, Gianfrilli D, Kanakis GA. Cardiovascular complications in patients with Klinefelter's syndrome. *Curr Pharm Des*. 2020;26(43):5556-5563.
304. Lincoff AM, Bhasin S, Flevaris P, *et al.* Cardiovascular safety of testosterone-replacement therapy. *N Engl J Med*. 2023;389(2):107-117.
305. Chang S, Christiansen CF, Bojesen A, Juul S, Münster A-MB, Gravholt CH. Klinefelter syndrome and testosterone treatment: a national cohort study on thrombosis risk. *Endocr Connect*. 2020;9(1):34-43.
306. Hussein TM, Abd Elmoaty Elneily D, Mohamed Abdelfattah Elsayed F, El-Attar LM. Genetic risk factors for venous thromboembolism among infertile men with Klinefelter syndrome. *J Clin Transl Endocrinol*. 2020;20:100228.
307. Chang S, Biltoft D, Skakkebæk A, *et al.* Testosterone treatment and association with thrombin generation and coagulation inhibition in Klinefelter syndrome: a cross-sectional study. *Thromb Res*. 2019;182:175-181.
308. Indirli R, Ferrante E, Scalambrino E, *et al.* Procoagulant imbalance in Klinefelter syndrome assessed by thrombin generation assay and whole-blood thromboelastometry. *J Clin Endocrinol Metab*. 2021;106(4):e1660-e1672.
309. Jin H, Lin J, Fu L, *et al.* Physiological testosterone stimulates tissue plasminogen activator and tissue factor pathway inhibitor and inhibits plasminogen activator inhibitor type 1 release in endothelial cells. *Biochem Cell Biol*. 2007;85(2):246-251.
310. Samango-Sprouse C, Stapleton E, Chea S, *et al.* International investigation of neurocognitive and behavioral phenotype in 47, XXY (Klinefelter syndrome): predicting individual differences. *Am J Med Genet A*. 2018;176(4):877-885.
311. Ross JL, Roeltgen DP, Kushner H, *et al.* Behavioral and social phenotypes in boys with 47,XYY syndrome or 47,XXY Klinefelter syndrome. *Pediatrics*. 2012;129(4):769-778.
312. van Rijn S, Bierman M, Bruining H, Swaab H. Vulnerability for autism traits in boys and men with an extra X chromosome (47, XXY): the mediating role of cognitive flexibility. *J Psychiatr Res*. 2012;46(10):1300-1306.
313. Esterman M, Rothlein D. Models of sustained attention. *Curr Opin Psychol*. 2019;29:174-180.
314. Ross JL, Zeger MPD, Kushner H, Zinn AR, Roeltgen DP. An extra X or Y chromosome: contrasting the cognitive and motor phenotypes in childhood in boys with 47,XYY syndrome or 47,XXY Klinefelter syndrome. *Dev Disabil Res Rev*. 2009;15(4):309-317.
315. van Rijn S, Swaab H. Executive dysfunction and the relation with behavioral problems in children with 47,XXY and 47,XXX. *Genes Brain Behav*. 2015;14(2):200-208.
316. Janusz J, Harrison C, Boada C, *et al.* Executive function in XXY: comparison of performance-based measures and rating scales. *Am J Med Genet C Semin Med Genet*. 2020;184(2):469-481.
317. Foland-Ross LC, Ghasemi E, Wun VL, *et al.* Executive dysfunction in Klinefelter syndrome: associations with brain activation and testicular failure. *J Clin Endocrinol Metab*. 2023;109(1):e88-e95.
318. Tartaglia N, Cordeiro L, Howell S, Wilson R, Janusz J. The spectrum of the behavioral phenotype in boys and adolescents 47, XXY (Klinefelter syndrome) 2010;8 Suppl 1(01):151-159.
319. Kuiper K, Swaab H, Tartaglia N, van Rijn S. Early developmental impact of sex chromosome trisomies on attention deficit-hyperactivity disorder symptomology in young children. *Am J Med Genet A*. 2021;185(12):3664-3674.
320. Temple CM, Sanfilippo PM. Executive skills in Klinefelter's syndrome. *Neuropsychologia*. 2003;41(11):1547-1559.
321. Kompus K, Westerhausen R, Nilsson LG, *et al.* Deficits in inhibitory executive functions in Klinefelter (47, XXY) syndrome. *Psychiatry Res*. 2011;189(1):135-140.
322. Fales CL, Knowlton BJ, Holyoak KJ, Geschwind DH, Swerdloff RS, Gonzalo IG. Working memory and relational reasoning in Klinefelter syndrome. *J Int Neuropsychol Soc*. 2003;9(6):839-846.
323. Green T, Flash S, Shankar G, *et al.* Effect of sex chromosome number variation on attention-deficit/hyperactivity disorder symptoms, executive function, and processing speed. *Dev Med Child Neurol*. 2022;64(3):331-339.
324. Bender BG, Linden MG, Robinson A. Neuropsychological impairment in 42 adolescents with sex chromosome abnormalities. *Am J Med Genet*. 1993;48(3):169-173.
325. Bender BG, Linden MG, Harmon RJ. Neuropsychological and functional cognitive skills of 35 unselected adults with sex chromosome abnormalities. *Am J Med Genet*. 2001;102(4):309-313.
326. Soto EF, Kofler MJ, Singh LJ, *et al.* Executive functioning rating scales: ecologically valid or construct invalid? *Neuropsychology*. 2020;34(6):605-619.
327. Isquith PK, Gioia GA, Espy KA. Executive function in preschool children: examination through everyday behavior. *Dev Neuropsychol*. 2004;26(1):403-422.
328. Jordan TL, Foland-Ross LC, Wun VL, Ross JL, Reiss AL. Cognition, academic achievement, adaptive behavior, and quality of life in child and adolescent boys with Klinefelter syndrome. *J Dev Behav Pediatr* 2023;44(7):e476-e485.

329. Kuiper KC, Swaab H, Tartaglia N, van Buggenhout G, Wouters C, van Rijn S. The developmental impact of sex chromosome trisomies on emerging executive functions in young children: evidence from neurocognitive tests and daily life skills. *Genes Brain Behav*. 2022;21(6):e12811.
330. Lee NR, Anand P, Will E, *et al.* Everyday executive functions in down syndrome from early childhood to young adulthood: evidence for both unique and shared characteristics compared to youth with sex chromosome trisomy (XXX and XXY). *Front Behav Neurosci*. 2015;9:264.
331. Van Rijn S. A review of neurocognitive functioning and risk for psychopathology in sex chromosome trisomy (47,XXY, 47, XXX, 47, XYY). *Curr Opin Psychiatry*. 2019;32(2):79-84.
332. Polanczyk G, de Lima MS, Horta BL, Biederman J, Rohde LA. The worldwide prevalence of ADHD: a systematic review and metaregression analysis. *Am J Psychiatry*. 2007;164(6):942-948.
333. Thomas R, Sanders S, Doust J, Beller E, Glasziou P. Prevalence of attention-deficit/hyperactivity disorder: a systematic review and meta-analysis. *Pediatrics*. 2015;135(4):e994-e1001.
334. Cederlöf M, Gotby AO, Larsson H, *et al.* Klinefelter syndrome and risk of psychosis, autism and ADHD. *J Psychiatr Res*. 2014;48(1):128-130.
335. Bruining H, Swaab H, Kas M, Van Engeland H. Psychiatric characteristics in a self-selected sample of boys with Klinefelter syndrome. *Pediatrics*. 2009;123(5):e865-e870.
336. Cordeiro L, Tartaglia N, Roeltgen D, Ross J. Social deficits in male children and adolescents with sex chromosome aneuploidy: a comparison of XXY, XYY, and XXYY syndromes. *Res Dev Disabil*. 2012;33(4):1254-1263.
337. Lee NR, Wallace GL, Clasen LS, *et al.* Executive function in young males with Klinefelter (XXY) syndrome with and without comorbid attention-deficit/hyperactivity disorder. *J Int Neuropsychol Soc*. 2011;17(3):522-530.
338. Moffitt TE, Arseneault L, Belsky D, *et al.* A gradient of childhood self-control predicts health, wealth, and public safety. *Proc Natl Acad Sci U S A*. 2011;108(7):2693-2698.
339. Shokrkon A, Nicoladis E. The directionality of the relationship between executive functions and language skills: a literature review. *Front Psychol*. 2022;13:848696.
340. Hamzik MP, Gropman AL, Brooks MR, Powell S, Sadeghin T, Samango-Sprouse CA. The effect of hormonal therapy on the behavioral outcomes in 47,XXY (Klinefelter syndrome) between 7 and 12 years of age. *Genes (Basel)*. 2023;14(7):1402.
341. Hinshaw SP, Nguyen PT, O'Grady SM, Rosenthal EA. Annual research review: attention-deficit/hyperactivity disorder in girls and women: underrepresentation, longitudinal processes, and key directions. *J Child Psychol Psychiatry*. 2022;63(4):484-496.
342. Haka-Ikse K, Stewart DA, Cripps MH. Early development of children with sex chromosome aberrations. *Pediatrics*. 1978;62(5):761-766.
343. Boada R, Janusz J, Hutaff-Lee C, Tartaglia N. The cognitive phenotype in Klinefelter syndrome: a review of the literature including genetic and hormonal factors. *Dev Disabil Res Rev*. 2009;15(4):284-294.
344. Leggett V, Jacobs P, Nation K, Scerif G, Bishop DVM. Neurocognitive outcomes of individuals with a sex chromosome trisomy: XXX, XYY, or XXY: a systematic review. *Dev Med Child Neurol*. 2010;52(2):119-129.
345. Zampini L, Draghi L, Silibello G, *et al.* Vocal and gestural productions of 24-month-old children with sex chromosome trisomies. *Int J Lang Commun Disord*. 2018;53(1):171-181.
346. Bishop DVM, Jacobs PA, Lachlan K, *et al.* Autism, language and communication in children with sex chromosome trisomies. *Arch Dis Child*. 2011;96(10):954-959.
347. Karipidis II, Hong DS. Specific learning disorders in sex chromosome aneuploidies: neural circuits of literacy and mathematics. *Am J Med Genet C Semin Med Genet*. 2020;184(2):518-530.
348. Graham JM Jr, Bashir AS, Stark RE, Silbert A, Walzer S. Oral and written language abilities of XXY boys: implications for anticipatory guidance. *Pediatrics*. 1988;81(6):795-806.
349. Boone KB, Swerdloff RS, Miller BL, *et al.* Neuropsychological profiles of adults with Klinefelter syndrome. *J Int Neuropsychol Soc*. 2001;7(4):446-456.
350. Mazzocco MMM. When a genetic disorder is associated with learning disabilities. In: *Neurogenetic Developmental Disorders*. The MIT Press; 2007:415-36.
351. Urbanus E, van Rijn S, Swaab H. A review of neurocognitive functioning of children with sex chromosome trisomies: identifying targets for early intervention. *Clin Genet*. 2020;97(1):156-167.
352. Butler G, Srirangalingam U, Faithfull J, Sangster P, Senniappan S, Mitchell R. Klinefelter syndrome: going beyond the diagnosis. *Arch Dis Child*. 2023;108(3):166-171.
353. Skakkebæk A, Wallentin M, Gravholt CH. Neuropsychology and socioeconomic aspects of Klinefelter syndrome: new developments. *Curr Opin Endocrinol Diabetes Obes*. 2015;22(3):209-216.
354. Ratcliffe S. Long-term outcome in children of sex chromosome abnormalities. *Arch Dis Child*. 1999;80(2):192-195.
355. van Rijn S, Stockmann L, van Buggenhout G, van Ravenswaaij-Arts C, Swaab H. Social cognition and underlying cognitive mechanisms in children with an extra X chromosome: a comparison with autism spectrum disorder. *Genes Brain Behav*. 2014;13(5):459-467.
356. Bender BG, Puck MH, Salbenblatt JA, Robinson A. Dyslexia in 47,XXY boys identified at birth. *Behav Genet*. 1986;16(3):343-354.
357. Rovet J, Netley C, Bailey J, Keenan M, Stewart D. Intelligence and achievement in children with extra X aneuploidy: a longitudinal perspective. *Am J Med Genet*. 1995;60(5):356-363.
358. Samango-Sprouse CA, Hamzik MP, Khaksari K, Brooks MR, Sadeghin T, Gropman AL. Novel neurocognitive profile in a minority of boys with 47,XXY (Klinefelter syndrome). *J Dev Behav Pediatr*. 2022;43(9):e623-e628.
359. Rovet J, Netley C, Keenan M, Bailey J, Stewart D. The psychoeducational profile of boys with Klinefelter syndrome. *J Learn Disabil*. 1996;29(2):180-196.
360. Geschwind DH, Boone KB, Miller BL, Swerdloff RS. Neurobehavioral phenotype of Klinefelter syndrome. *Ment Retard Dev Disabil Res Rev*. 2000;6(2):107-116.
361. Close S, Fennoy I, Smaldone A, Reame N. Phenotype and adverse quality of life in boys with Klinefelter syndrome. *J Pediatr*. 2015;167(3):650-657.
362. Sunil AB, Banerjee A, Divya M, Rathod HK, Patel J, Gupta M. Dyslexia: an invisible disability or different ability. *Ind Psychiatry J*. 2023;32(Suppl 1):S72-S75.
363. Wagner RK, Zirps FA, Edwards AA, *et al.* The prevalence of dyslexia: a new approach to its estimation. *J Learn Disabil*. 2020;53(5):354-365.
364. Brooks MR, Gropman AL, Hamzik MP, *et al.* Reading skills in males with 47,XXY: risk factors and the influence of hormonal replacement therapy (HRT). *Genet Med*. 2023;25(8):100864.
365. Samango-Sprouse CA, Stapleton EJ, Mitchell FL, Sadeghin T, Donahue TP, Gropman AL. Expanding the phenotypic profile of boys with 47, XXY: the impact of familial learning disabilities. *Am J Med Genet A*. 2014;164A(6):1464-1469.
366. Bojesen A, Stochholm K, Juul S, Gravholt CH. Socioeconomic trajectories affect mortality in klinefelter syndrome. *J Clin Endocrinol Metab*. 2011;96(7):2098-2104.
367. Martin S, Cordeiro L, Richardson P, Davis S, Tartaglia N. The association of motor skills and adaptive functioning in XXY/Klinefelter and XXYY syndromes. *Phys Occup Ther Pediatr*. 2019;39(4):446-459.
368. Samango-Sprouse CA, Tran SL, Lasutschinkow PC, *et al.* Neurodevelopmental outcome of prenatally diagnosed boys with 47,XXY (Klinefelter syndrome) and the potential influence of

early hormonal therapy. *Am J Med Genet A*. 2020;182(8):1881-1889.
369. Dulac O, Lassonde M, Sarnat HB. Pediatric neurology. *Handb Clin Neurol*. 2013;111:ix.
370. Visootsak J, Graham JM. Social function in multiple X and Y chromosome disorders: XXY, XYY, XXYY, XXXY. *Dev Disabil Res Rev*. 2009;15(4):328-332.
371. van Rijn S, Barendse M, van Goozen S, Swaab H. Social attention, affective arousal and empathy in men with Klinefelter syndrome (47,XXY): evidence from eyetracking and skin conductance. *PLoS One*. 2014;9(1):e84721.
372. van Rijn S, Swaab H, Aleman A, Kahn RS. X chromosomal effects on social cognitive processing and emotion regulation: a study with Klinefelter men (47,XXY). *Schizophr Res*. 2006;84(2-3):194-203.
373. van Rijn S, Aleman A, Swaab H, Krijn T, Vingerhoets G, Kahn RS. What it is said versus how it is said: comprehension of affective prosody in men with Klinefelter (47,XXY) syndrome. *J Int Neuropsychol Soc*. 2007;13(6):1065-1070.
374. van Rijn S, de Sonneville L, Swaab H. The nature of social cognitive deficits in children and adults with Klinefelter syndrome (47,XXY). *Genes Brain Behav*. 2018;17(6):e12465.
375. Bouw N, Swaab H, Tartaglia N, Cordeiro L, van Rijn S. Early social behavior in young children with sex chromosome trisomies (XXX, XXY, XYY): profiles of observed social interactions and social impairments associated with Autism Spectrum Disorder (ASD). *J Autism Dev Disord*. 2023;53(8):3194-3207.
376. van Rijn S. Social attention in 47,XXY (Klinefelter syndrome): visual scanning of facial expressions using eyetracking. *J Int Neuropsychol Soc*. 2015;21(5):364-372.
377. Urbanus E, Swaab H, Tartaglia N, van Rijn S. Social communication in young children with sex chromosome trisomy (XXY, XXX, XYY): a study with eye tracking and heart rate measures. *Arch Clin Neuropsychol*. 2023;39(4):482-497.
378. Bouw N, Swaab H, Tartaglia N, Wilson RL, Velde K, van Rijn S. Early symptoms of autism spectrum disorder (ASD) in 1–8 year old children with sex chromosome trisomies (XXX, XXY, XYY), and the predictive value of joint attention. *Eur Child Adolesc Psychiatry*. 2023;32(11):2323-2334.
379. van Rijn S, Swaab H, Aleman A, Kahn RS. Social behavior and autism traits in a sex chromosomal disorder: Klinefelter (47XXY) syndrome. *J Autism Dev Disord*. 2008;38(9):1634-1641.
380. van Rijn S, Stockmann L, Borghgraef M, *et al*. The social behavioral phenotype in boys and girls with an extra X chromosome (Klinefelter syndrome and trisomy X): a comparison with autism spectrum disorder. *J Autism Dev Disord*. 2014;44(2):310-320.
381. Skakkebæk A, Moore PJ, Pedersen AD, *et al*. The role of genes, intelligence, personality, and social engagement in cognitive performance in Klinefelter syndrome. *Brain Behav*. 2017;7(3):e00645.
382. Neuhaus E, Bernier RA, Webb SJ. Social motivation across multiple measures: caregiver-report of children with autism spectrum disorder. *Autism Res*. 2021;14(2):369-379.
383. Martin F, Swaab H, Bierman M, van Rijn S. Effectiveness of social management training on executive functions in males with Klinefelter syndrome (47, XXY). *Appl Neuropsychol Adult*. Published online December 10, 2023. Doi: 10.1080/23279095.2023.2262659.
384. Gross JJ. The emerging field of emotion regulation: an integrative review. *Rev Gen Psychol*. 1998;2(3):271-299.
385. Aldao A, Nolen-Hoeksema S, Schweizer S. Emotion-regulation strategies across psychopathology: a meta-analytic review. *Clin Psychol Rev*. 2010;30(2):217-237.
386. van Rijn S, Swaab H. Emotion regulation in adults with Klinefelter syndrome (47,XXY): neurocognitive underpinnings and associations with mental health problems. *J Clin Psychol*. 2020;76(1):228-238.
387. Boks MPM, de Vette MHT, Sommer IE, *et al*. Psychiatric morbidity and X-chromosomal origin in a Klinefelter sample. *Schizophr Res*. 2007;93(1-3):399-402.
388. Turriff A, Levy HP, Biesecker B. Prevalence and psychosocial correlates of depressive symptoms among adolescents and adults with Klinefelter syndrome. *Genet Med*. 2011;13(11):966-972.
389. DeLisi LE, Maurizio AM, Svetina C, *et al*. Klinefelter's syndrome (XXY) as a genetic model for psychotic disorders. *Am J Med Genet B Neuropsychiatr Genet*. 2005;135B(1):15-23.
390. Delavenne H, Khoury JM, Thibaut F, Garcia FD. CASE-REPORT Case study of a patient presenting both type II bipolar affective disorder and Klinefelter syndrome. *Genet Mol Res*. 2016;15(4).
391. Verhoeven WMA, Egger JIM. Schizotypy: key feature of Klinefelter's syndrome? *BMJ Case Rep*. 2011;2011:bcr0520114279.
392. Warwick MM, Lawrie SM, Beveridge A, Johnstone EC. Abnormal cerebral asymmetry and schizophrenia in a subject with Klinefelter's syndrome (XXY). *Biol Psychiatry*. 2003;53(7):627-629.
393. Kawahara K, Jono T, Nishi Y, Ushijima H, Ikeda M. Effects of testosterone therapy on bipolar disorder with Klinefelter syndrome. *Gen Hosp Psychiatry*. 2015;37(2):192.e1-192.e2.
394. Mors O, Mortensen PB, Ewald H. No evidence of increased risk for schizophrenia or bipolar affective disorder in persons with aneuploidies of the sex chromosomes. *Psychol Med*. 2001;31(3):425-430.
395. Turriff A, Levy HP, Biesecker B. Factors associated with adaptation to Klinefelter syndrome: the experience of adolescents and adults. *Patient Educ Couns*. 2015;98(1):90-95.
396. Schröder J, de la Chapelle A, Hakola P, Virkkunen M. The frequency of XYY and XXY men among criminal offenders. *Acta Psychiatr Scand*. 1981;63(3):272-276.
397. Witkin HA, Mednick SA, Schulsinger F, *et al*. Criminality in XYY and XXY men. *Science*. 1976;193(4253):547-555.
398. Nielsen J. Criminality among patients with Klinefelter's syndrome and the XYY syndrome. *Br J Psychiatry*. 1970;117(539):365-369.
399. Stochholm K, Juul S, Gravholt CH. Socio-economic factors affect mortality in 47,XYY syndrome-A comparison with the background population and Klinefelter syndrome. *Am J Med Genet A*. 2012;158A(10):2421-2429.
400. Herlihy AS, McLachlan RI, Gillam L, Cock ML, Collins V, Halliday JL. The psychosocial impact of Klinefelter syndrome and factors influencing quality of life. *Genet Med*. 2011;13(7):632-642.
401. Fjermestad KW, Stokke S. Sleep problems and life satisfaction as predictors of health in men with sex chromosome aneuploidies. *Behav Med*. 2018;44(2):116-122.
402. Bojesen A, Juul S, Birkebaek N, Gravholt CH. Increased mortality in Klinefelter syndrome. *J Clin Endocrinol Metab*. 2004;89(8):3830-3834.
403. Turriff A, Macnamara E, Levy HP, Biesecker B. The impact of living with Klinefelter syndrome: a qualitative exploration of adolescents and adults. *J Genet Couns*. 2017;26(4):728-737.
404. Franik S, Fleischer K, Kortmann B, *et al*. Quality of life in men with Klinefelter syndrome: a multicentre study. *Endocr Connect*. 2023;12(10):e230111.
405. Fjermestad KW, Huster R, Thunberg C, Stokke S, Gravholt CH, Solbakk AK. Neuropsychological functions, sleep, and mental health in adults with Klinefelter syndrome. *Am J Med Genet C Semin Med Genet*. 2020;184(2):482-492.
406. Fabrazzo M, Accardo G, Abbondandolo I, *et al*. Quality of life in Klinefelter patients on testosterone replacement therapy compared to healthy controls: an observational study on the impact of psychological distress, personality traits, and coping strategies. *J Endocrinol Invest*. 2021;44(5):1053-1063.
407. de Ronde W, de Haan A, Drent ML. Quality of life is reduced in patients with Klinefelter syndrome on androgen replacement therapy. *Eur J Endocrinol*. 2009;160(3):465-468.

408. Swerdlow AJ, Schoemaker MJ, Higgins CD, Wright AF, Jacobs PA; UK Clinical Cytogenetics Group. Cancer incidence and mortality in men with Klinefelter syndrome: a cohort study. *J Natl Cancer Inst*. 2005;97(16):1204-1210.
409. Hasle H, Mellemgaard A, Nielsen J, Hansen J. Cancer incidence in men with Klinefelter syndrome. *Br J Cancer*. 1995;71(2):416-420.
410. Ji J, Zöller B, Sundquist J, Sundquist K. Risk of solid tumors and hematological malignancy in persons with Turner and Klinefelter syndromes: a national cohort study. *Int J Cancer*. 2016;139(4):754-758.
411. Dyba T, Randi G, Bray F, *et al*. The European cancer burden in 2020: incidence and mortality estimates for 40 countries and 25 major cancers. *Eur J Cancer*. 2021;157:308-347.
412. Ferzoco RM, Ruddy KJ. The epidemiology of male breast cancer. *Curr Oncol Rep*. 2016;18(1):1.
413. Brinton LA. Breast cancer risk among patients with Klinefelter syndrome. *Acta Paediatr*. 2011;100(6):814-818.
414. Williams LA, Pankratz N, Lane J, *et al*. Klinefelter syndrome in males with germ cell tumors: a report from the Children's Oncology Group. *Cancer*. 2018;124(19):3900-3908.
415. Sawalha AH, Harley JB, Scofield RH. Autoimmunity and Klinefelter's syndrome: when men have two X chromosomes. *J Autoimmun*. 2009;33(1):31-34.
416. Scofield RH, Bruner GR, Namjou B, *et al*. Klinefelter's syndrome (47,XXY) in male systemic lupus erythematosus patients: support for the notion of a gene-dose effect from the X chromosome. *Arthritis Rheum*. 2008;58(8):2511-2517.
417. Frederick R, Vuillermin P, Tang MLK, *et al*. A child with Klinefelter syndrome and both IgE-mediated food allergy and low proportion of naive Treg. *Clin Case Rep*. 2019;7(2):302-303.
418. Ghisa M, Savarino V, Buda A, Katzka DA, Savarino E. Toward a potential association between eosinophilic esophagitis and Klinefelter syndrome: a case series and review of the literature. *Therap Adv Gastroenterol*. 2022;15:17562848221076888.
419. Panimolle F, Tiberti C, Granato S, *et al*. Screening of endocrine organ-specific humoral autoimmunity in 47,XXY Klinefelter's syndrome reveals a significant increase in diabetes-specific immunoreactivity in comparison with healthy control men. *Endocrine*. 2016;52(1):157-164.
420. Panimolle F, Tiberti C, Spaziani M, *et al*. Non-organ-specific autoimmunity in adult 47,XXY Klinefelter patients and higher-grade X-chromosome aneuploidies. *Clin Exp Immunol*. 2021;205(3):316-325.
421. Tarani L, Ceci FM, Carito V, *et al*. Neuroimmune dysregulation in prepubertal and adolescent individuals affected by Klinefelter syndrome. *Endocr Metab Immune Disord Drug Targets*. 2023;23(1):105-114.
422. Koçar IH, Yesilova Z, Ozata M, Turan M, Sengül A, Ozdemir I. The effect of testosterone replacement treatment on immunological features of patients with Klinefelter's syndrome. *Clin Exp Immunol*. 2000;121(3):448-452.
423. Bianchi I, Lleo A, Gershwin ME, Invernizzi P. The X chromosome and immune associated genes. *J Autoimmun*. 2012;38(2-3):J187-J192.
424. Gravholt CH, Andersen NH, Christin-Maitre S, *et al*. Clinical practice guidelines for the care of girls and women with Turner syndrome. *Eur J Endocrinol*. 2024;190(6):G53-G151.
425. Syrett CM, Sierra I, Beethem ZT, Dubin AH, Anguera MC. Loss of epigenetic modifications on the inactive X chromosome and sex-biased gene expression profiles in B cells from NZB/W F1 mice with lupus-like disease. *J Autoimmun*. 2020;107:102357.
426. Bellott DW, Hughes JF, Skaletsky H, *et al*. Mammalian Y chromosomes retain widely expressed dosage-sensitive regulators. *Nature*. 2014;508(7497):494-499.
427. Brown CJ, Ballabio A, Rupert JL, *et al*. A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome. *Nature*. 1991;349(6304):38-44.
428. Dou DR, Zhao Y, Belk JA, *et al*. Xist ribonucleoproteins promote female sex-biased autoimmunity. *Cell*. 2024;187(3):733-749.e16.
429. Zollner TM, Veraart JC, Wolter M, *et al*. Leg ulcers in Klinefelter's syndrome—further evidence for an involvement of plasminogen activator inhibitor-1. *Br J Dermatol*. 1997;136(3):341-344.
430. Isidori AM, Aversa A, Calogero A, *et al*. Adult- and late-onset male hypogonadism: the clinical practice guidelines of the Italian Society of Andrology and Sexual Medicine (SIAMS) and the Italian Society of Endocrinology (SIE). *J Endocrinol Invest*. 2022;45(12):2385-2403.
431. Davis SM, Lahlou N, Cox-Martin M, Kowal K, Zeitler PS, Ross JL. Oxandrolone treatment results in an increased risk of gonadarche in prepubertal boys with Klinefelter syndrome. *J Clin Endocrinol Metab*. 2018;103(9):3449-3455.
432. Ross JL, Kushner H, Kowal K, *et al*. Androgen treatment effects on motor function, cognition, and behavior in boys with Klinefelter syndrome. *J Pediatr*. 2017;185:193-199.e4.
433. Berger C, Goltzman D, Langsetmo L, *et al*. Peak bone mass from longitudinal data: implications for the prevalence, pathophysiology, and diagnosis of osteoporosis. *J Bone Miner Res*. 2010;25(9):1948-1957.
434. Lindgren E, Rosengren BE, Karlsson MK. Does peak bone mass correlate with peak bone strength? Cross-sectional normative dual energy X-ray absorptiometry data in 1052 men aged 18–28 years. *BMC Musculoskelet Disord*. 2019;20(1):404.
435. Lucas-Herald AK, Alves-Lopes R, Montezano AC, Ahmed SF, Touyz RM. Genomic and non-genomic effects of androgens in the cardiovascular system: clinical implications. *Clin Sci*. 2017;131(13):1405-1418.
436. Sesti F, Pofi R, Minnetti M, Tenuta M, Gianfrilli D, Isidori AM. Late-onset hypogonadism: reductio ad absurdum of the cardiovascular risk-benefit of testosterone replacement therapy. *Andrology*. 2020;8(6):1614-1627.
437. Corona G, Rastrelli G, Di Pasquale G, Sforza A, Mannucci E, Maggi M. Endogenous testosterone levels and cardiovascular risk: meta-analysis of observational studies. *J Sex Med*. 2018;15(9):1260-1271.
438. Bhasin S, Lincoff AM, Basaria S, *et al*. Effects of long-term testosterone treatment on cardiovascular outcomes in men with hypogonadism: rationale and design of the TRAVERSE study. *Am Heart J*. 2022;245:41-50.
439. Pencina KM, Travison TG, Artz AS, *et al*. Efficacy of testosterone replacement therapy in correcting anemia in men with hypogonadism: a randomized clinical trial. *JAMA Netw Open*. 2023;6(10):e2340030.
440. Bhasin S, Travison TG, Pencina KM, *et al*. Prostate safety events during testosterone replacement therapy in men with hypogonadism: a randomized clinical trial. *JAMA Netw Open*. 2023;6(12):e2348692.
441. Tsametis CP, Isidori AM. Testosterone replacement therapy: for whom, when and how? *Metabolism*. 2018;86:69-78.
442. Bartlett YK, Coulson NS. An investigation into the empowerment effects of using online support groups and how this affects health professional/patient communication. *Patient Educ Couns*. 2011;83(1):113-119.
443. Ahmed SF, Achermann J, Alderson J, *et al*. Society for endocrinology UK guidance on the initial evaluation of a suspected difference or disorder of sex development (revised 2021). *Clin Endocrinol*. 2021;95(6):818-840.
444. Close S, Smaldone A, Fennoy I, Reame N, Grey M. Using information technology and social networking for recruitment of research participants: experience from an exploratory study of pediatric Klinefelter syndrome. *J Med Internet Res*. 2013;15(3):e2286.
445. Sanders C, Hall J, Sanders C, *et al*. Involving individuals with disorders of sex development and their parents in exploring new models of shared learning: proceedings from a DSDnet COST action workshop. *Sex Dev*. 2018;12(5):225-231.
446. Kyriakou A, Dessens A, Bryce J, *et al*. Current models of care for disorders of sex development–results from an international survey of specialist centres. *Orphanet J Rare Dis*. 2016;11(1):155.

447. Werler S, Demond H, Damm OS, *et al.* Germ cell loss is associated with fading Lin28a expression in a mouse model for Klinefelter's syndrome. *Reproduction*. 2014;147(3):253-264.
448. Lue Y, Jentsch JD, Wang C, *et al.* XXY mice exhibit gonadal and behavioral phenotypes similar to Klinefelter syndrome. *Endocrinology*. 2005;146(9):4148-4154.
449. Eicher EM, Hale DW, Hunt PA, *et al.* The mouse Y* chromosome involves a complex rearrangement, including interstitial positioning of the pseudoautosomal region. *Cytogenet Genome Res*. 1991;57(4):221-230.
450. Wistuba J, Beumer C, Brehm R, Gromoll J. 41,XX(Y) * male mice: an animal model for Klinefelter syndrome. *Am J Med Genet C Semin Med Genet*. 2020;184(2):267-278.
451. Alowaysi M, Fiacco E, Astro V, Adamo A. Establishment of iPSC lines from a high-grade Klinefelter syndrome patient (49-XXXXY) and two genetically matched healthy relatives (KAUSTi003-A, KAUSTi004–A, KAUSTi004-B, KAUSTi005-A, KAUSTi005-B, KAUSTi005-C). *Stem Cell Res*. 2020;49:102008.
452. Astro V, Adamo A. Generation of iPSC cell lines from patients with sex chromosome aneuploidies. *Methods Mol Biol*. 2024;2770:185-200.
453. Botman O, Hibaoui Y, Giudice MG, *et al.* Modeling Klinefelter syndrome using induced pluripotent stem cells reveals impaired germ cell differentiation. *Front Cell Dev Biol*. 2020;8:567454.
454. Bojesen A, Gravholt CH. Morbidity and mortality in Klinefelter syndrome (47, XXY). *Acta Paediatr*. 2011;100(6):807-813.
455. Ali SR, Bryce J, Tan LE, *et al.* The EuRRECa project as a model for data access and governance policies for rare disease registries that collect clinical outcomes. *Int J Environ Res Public Health*. 2020;17(23):8743.
456. Williamson PR, Altman DG, Blazeby JM, *et al.* Developing core outcome sets for clinical trials: issues to consider. *Trials*. 2012;13(1):132.
457. Kodra Y, Weinbach J, Posada-de-la-Paz M, *et al.* Recommendations for improving the quality of rare disease registries. *Int J Environ Res Public Health*. 2018;15(8):1644.