Trends in Andrology and Sexual Medicine
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Andrea Garolla
Giovanni Corona
Editors

Klinefelter's Syndrome

From a Disabling Condition to a Variant of Normalcy

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Trends in Andrology and Sexual Medicine

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NOI
Noi siamo NOI
così simili a voi.
Fatti di carne,
di sangue e di anima.
Vogliamo che guardandoci
vediate voi stessi.
Che nel capirci complessi
ci vogliate completi.
Individui che nascono
e crescono accanto a voi,
Noi Klinefelter.
WE
We are US
so similar to you.
Meat made,
blood and soul.
We want you to look at us
see for yourself.
That in understanding complexes
you want us complete.
Individuals born
and grow next to you,
We Klinefelter.

Preface

Klinefelter syndrome (KS), both mosaic and non-mosaic forms, is the most frequent genetic condition with reproductive consequences that can be diagnosed initially or in childhood, adolescence, and adulthood. In relation to its great clinical variability, in many cases it even remains undiagnosed. Only a few decades ago, this condition was strongly associated with sterility, but more recently, parenthood options for KS have broadened significantly. Despite in recent decades it has become obvious that such a goal is highly unlikely with traditional therapy, fertility is often possible using assisted fertility techniques. Furthermore, a newer understanding of the syndrome instructs us to early recognize the affected patients and when to normalize the hormone imbalance, preventing health problems and improving quality of life. The impact of a comprehensive approach to KS brings to consider this condition as a variant of normal. However, additional conditions that may be associated with the syndrome such as obesity, reduced bone density, sexual or psychomotor abnormalities, and developmental dyspraxia represent concerns that induced to refuse this normal variant concept in the past.

The clinical andrologist has a key role in the diagnosis, management, treatment, and follow-up of KS. However, other specialists such as pediatricians, geneticists, psychiatrists, endocrinologists, psychologists, and speech therapists have central roles in particular conditions. In the current international literature, a book considering all the clinical aspects of KS is missing. Therefore, the Klinefelter Italian Group (KING) of the Italian Society of Andrology and Sexual Medicine (SIAMS), supported by the Springer books, asked to prominent Italian experts of the main specialties committed in KS, to develop a textbook using most recent available evidence. Editors, Andrea Garolla, coordinator of KING, and Giovanni Corona, president of SIAMS, included all the topics featured by experts in KS in this book. They considered epidemiology, counseling to family and patients, genetic and epigenetic factors, developmental problems, psychological features, fertility problems and preservation, sexual function, and possible comorbidities such as osteoporosis, obesity, dyslipidemia, altered glucose metabolism, thyroid dysfunction, cancer risk, cardiovascular problems, and strategies of management and treatment from birth to adulthood. Finally, they strongly wanted the presence of KS patients in the book and

included at the start a poetry depicting the feelings of an affected subject. Special thanks to all the authors and collaborators for their strong efforts and fruitful collaboration and for harmoniously integrating their competence.

Padova, Italy

Carlo Foresta
Andrea Garolla

Contents

Introduction: From a Disabling Condition to a Variant of Normalcy

1

Andrea Garolla

The syndrome was reported first by Klinefelter et al. in 1942. Authors described clinical conditions of a group of patients characterized by gynecomastia, azoospermia, hypogonadism, and high follicle stimulating hormone (FSH) plasma levels [1]. Only in 1959, with the introduction of genetics, a study published in the *Nature* journal clarified that these subjects have an extra X chromosome and that their karyotype is 47,XXY [2]. Klinefelter syndrome (KS) is the most frequent abnormality of sex chromosomes, with an estimated prevalence ranging from 1:500 to 1:700 in newborn males [3], therefore representing the most common chromosomal alteration in males [4]. Although most cases show a 47,XXY karyotype, some patients have mosaicisms (i.e., 46XY/47XXY) or chromosome aneuploidies with more than one extra X chromosome, such as 48XXXY and 49XXXXY [3].

The phenotype of Klinefelter subjects can be very different also in the presence of the same karyotype. In fact, affected patients may have both clear signs and symptoms related to chromosomal alteration or very little and even absent in some cases. Accordingly, it is common for the majority of Klinefelter subjects to remain undiagnosed or to have a late diagnosis during medical care for hypogonadism, couple infertility, and sexual dysfunction [4–6]. As reported in this book, it is clear that clinical conditions associated with the syndrome have very different incidence from one patient to another [7, 8]. In fact, these subjects may have or may have not the following problems: development, behavioral, psychological and learning problems, neuropsychiatric alterations, thyroid dysfunction, osteoporosis, abnormal lipids and glucose metabolism, cardiovascular impairment, oncological problems, and sexual dysfunction. Moreover, according to the literature, Klinefelter subjects may have a shortened life expectancy up to 2 years; however, they can still live a long, full life with this condition. In any case, although the phenotypic spectrum of the

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syndrome is wide, testicular dysfunction is a constant clinical finding, characterized by small testes, elevated gonadotropins from mid-puberty onward, and, with rare exceptions, absence of spermatozoa in the ejaculate [9].

Prenatally, the detection of 47,XXX karyotype is usually incidental during the screening for genetic abnormalities [10]. This diagnosis makes prenatal counseling and the parents' decision-making more complicated and challenging. In fact, a prenatal diagnosis affects parental perceptions and expectation for their child. As reported by some authors, the knowledge of medical problems possibly associated with Klinefelter syndrome, during prenatal counselling, raises major concerns to pregnant mothers and it is probably the cause of low rates of pregnancy termination and high voluntary abortions [11]. The type of medical professional providing the counseling, as well as the scientific literature presented, can significantly impact whether a termination takes place and the parents' expectation as their child develops [12]. Despite some authors described the increased association between prenatal identification of 47,XXY and preterm delivery or maternal anxiety [13], other studies reported the benefits of early diagnosis [14–16]. Based on a sensitive and comprehensive prenatal counseling, providing current information on neuropsychology, infertility, and neurodevelopmental progression associated with this disorder, families can be proactive and preventive by developing focused interventional strategies reflective of possible vulnerabilities of their Klinefelter child that could be longitudinally beneficial for minimizing the developing language-based disorders, graphomotor dysfunction, and social anxiety [17, 18].

Because of the many symptoms that are frequently related to the syndrome, Klinefelter patients are invariably considered as affected by a rare or chronic but, in any case, disabling condition. Even if in some cases this aspect may help patients to have a better health assistance, in other cases it could be considered as a handicap and lead to a social discrimination. This aspect represents a big problem for Klinefelter subjects; in fact, it is well known that people with the syndrome may have a normal social and family life, a normal job, and may reach the higher levels of study and working. Therefore, the criteria used to evaluate Klinefelter patients cannot be generalized. Each patient should be individually investigated to get a correct and tailored judgement of possible problems. In the light of these considerations and because any subject with no chromosomal alteration may complain of the same problems as Klinefelter subjects, this book aims to highlight that KS has to be considered a variant of normalcy more than a disabling condition.

In conclusion, we suggest that the prenatal counseling of families with pregnancies affected by fetal 47,XXY and the evaluation of patients with KS are provided by experts of this condition. This approach will provide the chance to avoid termination of Klinefelter embryos and to evaluate and manage in a correct manner all possible health problems of affected patients. Moreover, the final judgement on the state of health of patients should not be based on the syndrome but on the individual condition. This approach will allow the scientific community to change the point of view on KS and to consider this condition as a variant of normalcy.

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Epidemiology of an Underdiagnosed Syndrome

2

Marco Zavattaro, Lorenzo Marinelli, Giovanna Motta, and Fabio Lanfranco

2.1 Difficulties in Prevalence Estimation

The diagnosis of a KS male is based on the clinical appearance coupled with a karyotype finding of 47,XXY or mosaics thereof (usually with a 47,XXY/46,XY karyotype). People with additional sex chromosomes (48,XXXY, 48,XXYY and other polysomies) should not be considered KS males because they usually have a much more affected phenotype [1]. There is no universal agreement on the main clinical signs or stigmata that should lead to karyotyping [2, 3]. The absence of overt clinical signs makes it difficult to distinguish many males with KS from 46,XY male [3, 4] and this represents one of the main determinants for the underdiagnosis of the disease. These aspects are even more significant in patients with mosaicism (up to 20% of KS cases) since they may present larger testicular volume, more frequently sperm in the ejaculate, and higher androgen levels than their non-mosaic counterpart [5]. Moreover, chromosomal mosaicism can be present only in the testes, leading to a normal result on karyotype analysis of peripheral leukocytes.

Several studies indicate that only from 25% to 40% KS males are ever diagnosed [6–8] and only about 10% of them are diagnosed during childhood and adolescence. The majority of patients are diagnosed during adulthood, typically in the course of an infertility workup, with a mean age of 27 years, according to updated Danish data [7].

However, the modern genetics and laboratory techniques allow evaluation of aneuploidies even prenatally or in early pregnancy by amniocentesis, chorionic villus sampling, or cell-free DNA testing [9]. In particular, cell-free DNA testing in maternal plasma is an examination technique suggested in mothers with a high risk

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for aneuploidies. Since its introduction, it has increased prenatal KS diagnosis, while the overall prenatal aneuploidy detection has remained stable [10].

The possibility of early diagnoses brings with it ethical issues, including those related to abortion. About 44–85% of mothers carrying a 47,XXY foetus decide for legal abortion, potentially limiting the estimation of the real miscarriage rate of 47,XXY implanted embryos and reducing post-natal prevalence of KS, as confirmed by data from the Danish national register [7, 11–13]. In fact, legal abortion is expected to slightly reduce the prevalence of live-born KS males from 150 to 140 per 100,000 males [7, 13]. However, after a prenatal diagnosis of KS, a multidisciplinary approach involving a geneticist and a psychologist proved to be effective in reducing the abortion rate (from 46.9% to 11.6%) [14].

Although the abortion rate seems to be only marginally affected by the low level of detection of prenatal KS, an optimization in diagnostic techniques (especially cell-free DNA testing) is mandatory. It could allow an early detection of the syndrome and its most appropriate management in a wider number of KS males.

From this perspective, a population-based neonatal genetic screening may represent the most accurate method to clarify several questions concerning prevalence and phenotypic heterogeneity of KS [15–17]. A neonatal screening may be particularly appropriate even if the real impact of early diagnoses in reducing long-term morbidity and mortality is still being debated [16, 17].

2.2 Prevalence of Klinefelter Syndrome

No precise data are currently available regarding the real prevalence of KS. Ten percent of KS males are detected prenatally, 3% are identified before the age of 20 due to developmental delays or behavioural problems, and only 2% are diagnosed due to delayed puberty or gynaecomastia. The corresponding proportion of cases diagnosed in adulthood due to hypogonadism or infertility is reported to be 17% [8]. As previously mentioned, this leaves the majority of KS undiagnosed. More recent studies [18, 19] report different percentages regarding the median age at diagnosis (21% detected prenatally, 28% pre-adulthood, 51% in adulthood).

At the current state of knowledge, the prenatal prevalence of KS varies between 0.2%, when evaluated through amniocentesis and chorionic villi sampling [7], and 0.9%, when considering human blastocysts analysed with preimplantation genetic testing for aneuploidies during IVF cycles [20]. At present, available data are scanty and do not allow the causes of this difference to be defined with certainty, despite some studies hypothesized lower implantation and higher miscarriage rate in 47 XXY blastocysts. A higher prevalence of KS in human blastocysts may also be attributable to advanced maternal age (especially ≥35 years), as frequently observed in infertile couples undergoing IVF [20, 21]. Based on these considerations, some authors hypothesized that the prevalence of KS may be increasing. In fact, the progressive rise in maternal and paternal age at the time of conception is associated with a higher risk of meiotic non-disjunctions during gamete formation and thus 47,XXY karyotype [22].

Regarding postnatal prevalence, the first investigations about this topic [23–30] were conducted in the USA, Canada, Europe (United Kingdom and Denmark) and Asia (Russia and Japan), stating a prevalence ranging from 85 to 223 per 100,000 males. These data were later confirmed by other studies [31–33]. In particular, in Denmark, where data from solid registries of newborns are available, authors estimated a prenatal prevalence of KS ranging from 153 to 173 per 100,000 males; this result is in line with the findings of a study performed in the early 1990s, which found a postnatal prevalence (in live-born males) of 152 per 100,000 males [7, 13, 30].

Interestingly, on a deeper analysis of these registries, it was found that only 25% of KS males were diagnosed in adulthood, leading to an estimated prevalence of 28–40 per 100,000 males, far fewer than expected [7, 13, 32]. These data were confirmed by studies from United Kingdom and Sweden that estimated a diagnostic rate of 11.9 and 23.1 per 100,000 males, respectively [34, 35].

These European data are not fully consistent with the Australian ones, which underlined a higher prevalence of KS males, both prenatally (223/100,000) and postnatally (87/100,000). The authors justify these findings due to the ethnic composition of the country (about 8% of the Australian population has an Asian descent compared to 3% of the Danish population) and with a mean older age of women who plan to become pregnant [36].

These findings seem to be confirmed by an American study, which stated a higher prevalence of KS male in Asiatic versus white ethnicity (355 vs. 166 per 100,000, respectively). This study is consistent with investigations conducted before 2000, in which primarily Caucasian and Asian individuals were included [33].

Finally, it should be noticed that fewer prevalence data on KS mosaics are available. Although their scarcity, it is thought that mosaicism may occur in about 10% of all KS diagnoses, both at prenatal and postnatal evaluation [5, 13].

2.3 Conclusions

Although historically considered a rare condition, current data on prenatal or postnatal prevalence show that KS is not so uncommon as once thought. Actual discrepancies between prenatal and postnatal prevalence confirm that most patients are not correctly diagnosed, with potential detrimental long-term consequences in terms of morbidity and mortality due to lack of optimal management [37, 38].

Conflict of Interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Causes of Extra Chromosome(s)

3

Savina Dipresa and Andrea Garolla

3.1 Mechanism of the Supernumerary X Chromosome

A normal female egg contains one X chromosome and a normal male sperm contains either one X or one Y chromosome. At conception, together they create a fertilised egg with either 46,XY or 46,XX chromosomes. Most of the chromosomal disorders of offspring arise at meiosis of the parental gametes: a gamete from a 46,XX or 46,XY adult may acquire an extra normal chromosome, leading to a *full aneuploidy* in the conceptus, in term of trisomy, or leading to a monosomic offspring in case of a chromosome loss [1].

More than half cases of Klinefelter syndrome result from a paternal error during the first meiotic division (MI), and so the extra chromosome comes from the father [2]. Fathers of paternally originating KS may have marginally elevated levels of disomic XY sperm in comparison to fathers of maternally originating cases, possibly reflecting an inherent tendency among a small minority of these men to produce aneuploid sperm [3]. Woods et al. [4] report two brothers with XXY karyotype, the only known example of KS recurrence, in which the karyotype of both brothers reflects a paternal meiosis I error.

Approximately 40% of XXY arrangement is due to a maternal meiotic error. The majority of these errors occur in the first meiotic division (MI), advanced maternal age increasing only slightly the risk for the XXY chromosome count.

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This can happen when the primary gametocytes (cells with 46 chromosomes) undergo meiosis, the process by which they become sperms and eggs, mature gametes with haploid complement of 23 chromosomes. Two cell divisions (meiosis I and meiosis II) follow to produce the normal gamete. During meiosis I, chromosomes pair with their corresponding chromosomes (in women X chromosomes pair; in men the X and Y chromosomes pair) and exchange bits of the genetic material to ensure genetic variation in the gametes. After the exchange, the chromosomes separate.

Occasionally the Xs or the X chromosome and Y chromosome fail to pair and fail to exchange the genetic material, producing an egg with two Xs or a sperm with an X and a Y chromosome. A Klinefelter patient, 47,XXY, is conceived whenever a sperm having both an X and a Y chromosome fertilises a normal egg with a single X chromosome, or a normal sperm having a single Y fertilises an egg with two X chromosomes.

In males, meiosis starts at puberty. During meiosis I, the primary spermatocyte (46 chromosomes) gives rise to two secondary gametocytes (23 chromosomes in the double-chromatid state). In meiosis II, the chromosomes of the secondary gametocyte separate into their component chromatids, producing four spermatids. Each one contains a haploid set of chromosomes and matures into spermatozoa. But malsegregation is frequent at meiosis, leading to trisomic or monosomic human conceptions.

According to the classic description of malsegregation, in a chromosomally normal person, if the pair of homologs comprising a bivalent at meiosis I fails to separate (fails to disjoin), one daughter cell will have two of the chromosomes (trisomic conception) and the other will have none (monosomic conception). This is 2:0 segregation, with one gametocyte disomic and the other nullisomic for that homolog. Otherwise, nondisjunction may occur in meiosis II because of the failure of chromatids to separate. An alternative to the foregoing classic scenario is the modern description of malsegregation, a model focused on the precocious separation of chromatids during meiosis I that involves three sequential events: homologs fail to pair during meiosis I, then the univalents are prone to pre-divide, and finally the chromosomes segregate independently to the mature spermatocytes [5]. A process somewhat intermediate between these two mechanisms is the ‘achiasmate nondisjunction’, but without any recombination [6].

In spermatogenesis, predivision and achiasmate nondisjunction are equally important malsegregant mechanisms; spermatocyte aneuploidy may be more frequent than previously considered, but the existence of a postmeiotic checkpoint may exclude most aneuploidy spermatozoa from full maturation. Chromosome 21 and the X and Y are most prone to nondisjunction in the male, whether at meiosis I or II.

Another quite plausible model, apart from meiosis-based descriptions, is the gonadal mosaicism that could explain at least a part of the cases. According to this model, the error in the gamete had happened at a premeiotic stage (mitotic errors of gametocytes), and the parent is a gonadal mosaic for the aneuploidy. Some authors

suggest mitotic errors during gametocyte replications (in early embryogenesis), when the potential for error exists at each cell division [7].

Some XXY boys/men have a mosaic chromosome makeup. This means that some cells of their body contain the extra X chromosome and some cells have a normal number of sex chromosomes (one X and one Y). Chromosomal mosaicism is believed to be the consequence of a postmeiotic event. Error occurred in the very earliest days after conception when the embryo was developing. A patient with not all cells with the extra chromosome is likely to have a less severe phenotype with respect to someone with all tissue involved by the aneuploidy, even if the clinical characteristics are qualitatively similar.

There is also *partial aneuploidy*, of just a part of the chromosome, due to meiotic malsegregation in gametogenesis of a parent carrying a balanced rearrangement. When the parental karyotype is normal, partial aneuploidy of the conceptus is de novo and may have taken place at a meiotic division or at a premeiotic germ cell mitosis.

X chromosome aneuploidy is associated with little phenotypic abnormality compared with autosomal imbalance. This is because only one X in each cell needs to be fully active (the phenomenon called dosage compensation) and the detrimental effects of the extra X are mitigated by inactivating the additional X chromosome, maintaining in each cell just one active X. Some loci are not subject to inactivation, and this is likely the reason for the phenotypic abnormalities associated with the condition [8].

Boys with more than one additional X (e.g. 48,XXXY or 49,XXXXY) are less common than boys with 47,XXY makeup. They should not be considered a variant of Klinefelter syndrome considering their own unique physical and behavioural characteristics. 48,XXXY syndrome can come from either the mother or the father. It usually arises when a Y-bearing sperm fertilises a woman's egg carrying XXX chromosomes or when a sperm with an XXY chromosome makeup fertilise an egg with a single X chromosome. It is not well understood why the two extra X chromosome were incorporated. In case of 49,XXXXY, it is supposed that the extra X chromosomes come from the mother's egg cell that has retained more than one X chromosome. Normally each one X chromosome would be placed into four separate egg cells during cell division [9]. When an egg cell (46,XX) divides during meiosis I, it makes a copy of its chromosomes (becomes XXXX) and, as it divides, it shares them equally between the two resulting cells. These two cells then undergo meiosis II, and when they divide again, the chromosomes are shared 21 equally between the two resulting cells. This process produces four egg cells with a single X chromosome. When an egg cell carrying four X chromosomes is formed, all X chromosomes are kept in a single cell during both cell divisions (nondisjunction of meiosis I and II). There is no evidence of a maternal age effect for nondisjunction in case of 49,XXXXY. Different from nondisjunction for other chromosomes, which is usually associated with older mothers, it is not known why the X chromosomes fail to separate properly [10].

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Different Karyotypes, Same Disease?

4

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Not very long after the original description by Harry F. Klinefelter Jr. et al. in 1942 of the eponymous syndrome (KS) [1], characterized by an additional X chromosome in six male individuals, came the first reports by Ford et al. of a man harboring 48 chromosomes (possibly a 48,XXYY karyotype) [2] and by Fraccaro et al. of a child with a 49,XXXXY karyotype (although originally believed to possess an additional X chromosome plus a trisomy of both chromosomes 8 and 11), manifesting with ambiguous genitalia, congenital heart disease, and cognitive deficit [3].

Although specific signs and symptoms pertaining to conditions other than the classical KS were originally recognized, as well as their increased overall severity, it came to widespread use by many authors [4–11] to label every male patient with at least one supernumerary X chromosome as being affected by KS or a variant thereof. This association was mostly justified by the common findings of tall stature and hypergonadotropic hypogonadism in all these patients. Nonetheless, others have advocated these conditions to substantially differ from KS [12–15], and as such should be best referred to as high-grade aneuploidies of the sex chromosomes (HGA) presenting with a male phenotype.

A number of different karyotypes have been described to belong to the HGA family, namely, 48,XXXY, 48,XXYY, 48,XYYY, 49,XXXXY, 49,XXXYY, and 49XYYYY, each possibly presenting as pure or mosaic forms, which contributes to their phenotype heterogeneity. HGA are characterized by elevated, although variable, rarity, ranging in prevalence from 1:17,000 to 1:100,000 males [16–18], with some having only few cases reported in the literature, as compared to approximately 1:650 males for KS [19], although correct estimates are lacking.

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The 47,XYY syndrome, also known as Jacobs syndrome, in our opinion should not be regarded as a HGA for its higher prevalence, milder phenotype, and substantially different clinical picture, and therefore we will not discuss it; interested readers can refer to other specific works [20–25].

A feature invariably observed in patients affected by HGA is the development of a hypergonadotropic hypogonadism, in which testicular damage occurs earlier and in a more severe form than that in KS. Testicular dysfunction often manifests as impaired genital development (e.g., micropenis and cryptorchidism) and otherwise becomes apparent by pubertal age, while testicular histology in adults with 48,XXYY, 48,XXXY, and 49,XXXXY syndromes shows a similar, yet more diffuse, hyalinization and fibrosis of the seminiferous tubules, loss of germ cells, and Leydig cell hyperplasia as in KS [2, 3, 26].

To date, the only study evaluating the endocrine and metabolic phenotype of HGA in comparison with KS [12] showed an earlier increase in follicle-stimulating hormone (FSH) values in patients younger than 12 years, and higher gonadotropin values in the 12–20 years age range accompanied by lower total testosterone and inhibin B and higher sex hormone-binding globulin (SHBG) values, seemingly indicating an earlier testicular damage; these differences, however, were no longer present in adulthood. Prolactin values were also higher than those in KS in the age range 12–20 years and in adulthood, while a normal thyroid function was observed, although with an altered free triiodothyronine/free thyroxine ratio (fT3/fT4). The study of the metabolic profile showed higher total and LDL cholesterol in HGA patients compared with KS at all ages and lower, although not significantly, HDL cholesterol; no differences were observed in triglyceride levels and in the glucose metabolism of HGA patients.

Currently, there is no specific treatment protocol or consensus for testosterone replacement therapy in HGA, and therefore patients usually begin testosterone therapy when delayed puberty is evident by late pubertal onset or pubertal arrest or, more rarely, in adult life following a finding of reduced testosterone levels. Treatment regimens do not currently differ based on the specific genetic diagnosis. More research is needed to determine whether an earlier therapy start or a "priming" during infancy or pre-pubertally could be beneficial in ameliorating brain development and/or body composition, similarly to what is recently being evidenced in KS [27, 28].

HGA are generally associated with more pronounced phenotypic *abnormalities compared to KS and are* characterized by mild craniofacial dysmorphism, skeletal anomalies, significant developmental delays, neurological symptoms, and poor dentition [29, 30]. The more significant cognitive and behavioral differences observed in HGA when compared to KS may be explained by the increased gene expression [14]. The phenotype of all HGA is proposed to result from gene dosage effects, in which genes on the additional X and Y chromosomes that are not X inactivated are therefore expressed at higher levels than those in typical 46,XY males. For example, genes that are expressed from both the X and Y chromosomes, in HGA would be expressed twofold to threefold in comparison with a 46,XY male. Microarray studies in 47,XXY have identified genes that are differentially expressed compared with 46,XY [31], and differentially expressed genes were correlated with verbal cognitive skills in one study. However, the behavioral characteristics of individuals with

Table 4.1 Comparison of the characteristics of the three main HGA syndromes

	48, XXYY	48, XXXY	49, XXXXY
Prevalence	1:18.000–40.000 males	1:17.000–50.000 males	1:85.000–100.000 males
Origin	Paternal	Maternal or paternal	Maternal
Facial dysmorphism	Hypertelorism Epicanthus Cleft palate Taurodontism	Hypertelorism Flat nasal bridge	Hypertelorism Microcephaly Flat nasal bridge Cleft palate Bifid uvula
Skeletal malformation	Clinodactyly Radioulnar synostosis Pes planus Joint laxity	Clinodactyly Radioulnar synostosis	Clinodactyly Radioulnar synostosis Pes cavum Genu valgum
Visceral involvement	Cardiac malformations Renal dysplasia Inguinal hernias	Cardiac malformations Renal dysplasia Inguinal hernias	Cardiac malformations Inguinal hernias
FSIQ	60–80	40–75	20–60

FSIQ full scale intelligence quotient

sex chromosome tetrasomy and pentasomy are limited to case studies and usually lack standardized normative measures.

Effects on physical and mental development increase with the number of extra Xs, and each X is roughly estimated to reduce the overall intelligence quotient (IQ) by 15 points, with the language domain being the most affected one [32], and the behavioral phenotype frequently highlights attention problems, impulsivity/aggression, and mood instability [29].

Data from epidemiological studies have evidenced an important increase in mortality in HGA for all causes, with a standardized mortality ratio (SMR) of 2.1, specifically from chronic lower respiratory disease and congenital anomalies. An increased standardized incidence ratio (SIR = 32.6) and SMR (36.7) have been specifically reported in 48,XXYY patients for non-Hodgkin lymphoma [33], although the authors admit that these data need confirmation from future studies.

The real complete spectrum of different HGA remains to be fully elucidated in detail and their phenotype possibly depends on the severity of the expression of the genetic defect, androgen deficiency, and androgen receptor sensitivity [34, 35].

Table 4.1 compares the principal findings of the three better known and more frequent HGA, namely, 48,XXYY, 48,XXXY, and 49,XXXXY syndromes, which are discussed in greater detail below.

4.1 48,XXYY

The first patient affected by a 48,XXYY karyotype was possibly described by Ford [2] and the syndrome was then correctly identified by Muldal and Ockey [36]. Its prevalence is estimated at 1:18<000–40,000 males [16].

Three possible mechanisms explain the formation of the 48,XXYY karyotype: (1) two consecutive non-disjunctive events in meiosis I and II in a normal man that fertilize a normal oocyte [37–40], (2) a single non-disjunctive event in meiosis I in a man harboring a 47,XYY karyotype [41], and (3) non-disjunction during mitosis of a normal fertilized egg. Although 1–2% of spermatozoa contain aneuploid sex chromosomes, as compared to more than 20% in oocytes [42], only the paternal origin of the 48,XXYY syndrome via a triploid gamete ($X_pY_pY_p$) has been actually documented.

Mean age at diagnosis has been reported to be 7.7 ± 5.6 years in a cohort of 95 patients [43], later than more complex HGA syndromes (see below) and much earlier than the median age at diagnosis of KS (27 years), after exclusion of prenatal diagnoses [19].

Males with 48,XXYY show a mean adult height of 193 cm [43], typically exceeding males with KS. They have an eunuchoid habitus characterized by long legs, abdominal adiposity, micropenis, and very small and cryptorchid testes or ambiguous genitalia [44]. Patient's body weight can vary from underweight to obese and approximately 30% show gynecomastia [14].

Facial dysmorphism is present in all HGA syndromes; however, it is variable in degree and most frequent features include hypertelorism, epicanthus, and upslanting palpebral fissures. The most frequently reported characteristics include clinodactyly of the fifth finger (up to 70% of patients), pes planus and clubfoot, joint laxity and radioulnar synostosis, renal dysplasia and cardiac malformations, inguinal hernias, cleft palate, and short nail beds [45–48]. Taurodontism, frequent caries, thin enamel, and malocclusion are among the most frequent dental problems [49]. Similar to KS, a tremor with features reminiscent of essential tremor is present in some patients [50].

48,XXYY is typically associated with a higher degree of neuropsychiatric symptoms compared to KS. The full scale intelligence quotient (FSIQ) ranges from 60 to 80, with 10% of patients having an IQ above 80 [16]. Global developmental delay and severe expressive language disability are common, and verbal IQ is usually lower than performance IQ. Speech and motor delays vary from 75% to 92% and the latter usually associates with hypotonia and a mean age at independent ambulation of 18 months. Behavioral features range from shyness to aggressiveness based on several case reports [32].

Attention deficit–hyperactivity disorder (ADHD) is present in over 70% of patients, according to a study based on parent- and teacher-reported symptoms (Diagnostic and Statistical Manual of Mental Disorders, DSM IV criteria), while the prevalence of autism spectrum disorders is estimated at 34%, with most patients only mildly affected and showing difficulties in social skills and social interactions [51].

These patients are azoospermic and are considered infertile. Nowadays, modern surgical techniques in combination with the assisted reproduction technology (ART) offer the possibility of fatherhood even to patients with azoospermia, where spermatozoa can be recovered using (microdissection) testicular sperm extraction (TESE/micro-TESE). This approach, combined with Intra-Cytoplasmatic Sperm Injection

(ICSI), is already frequently employed in KS patients [52]. The ability of different factors in predicting the success of TESE have been evaluated, including testicular size, Y chromosome microdeletions, genetic abnormalities, gonadotropin, and sex hormone levels [53]; however, none have been shown to be useful in clinical practice [54, 55]. Recently, a case report has been published of a successful micro-TESE performed in a 30 year-old man non-mosaic 48,XXYY and his spouse was successfully pregnant with a normal karyotype embryo [56].

4.2 48,XXXY

Among HGA, the prevalence for 48,XXXY is estimated at 1:17,000–50,000 males [17, 18].

The possible mechanisms that explain the formation of the 48,XXXY karyotype have been reported in five cases: in two of these cases, the origin was successive non-disjunction in formation of the sperm fertilizing a normal female oocyte. The other three cases indicated double non-disjunction events during oogenesis [39].

Mean age at diagnosis has been reported from available case reports to vary from infancy to early adulthood [57]. Males with 48,XXXY show a mean adult height of 190 cm [43], typically exceeding males with KS. Phenotypically, these patients resemble those with 48,XXYY, although with a somewhat more severe phenotype. Frequent physical findings include ocular hypertelorism, flat nasal bridge, radioulnar synostosis, fifth finger clinodactyly, micropenis, and severe testicular hypotrophy (and cryptorchidism) with classic hypergonadotropic hypogonadism, while congenital malformations are similar to that of 48,XXYY and 49,XXXXY [4].

Their IQ is typically between 40 and 75 and all show some degree of learning disability, with severe verbal retardation in more than half of the patients. The assessment of mental capacities itself can be quite challenging because of the invariable presence of some language impairment in these patients. Behaviorally, patients frequently show immaturity consistent with their IQ level and are described as passive and pleasant, although aggressive outbursts are frequent as a result of mental deficits [8, 32, 58, 59].

4.3 49,XXXXY

Among HGA, the prevalence for this condition has been estimated at 1:85,000–100,000 males [17, 18]. Mean age at diagnosis was 4 months in a cohort of 20 patients [9] and this is coherent with the inherent gravity of this syndrome.

Genetically, 49,XXXXY seems to results from non-disjunction of the X chromosome during both meiosis I and II, that is, an aneuploid oocyte being fertilized with a normal male sperm [39, 60]. Although stature of these patients has been described in some case reports to be lower than other HGA tetrasomies, this is not always the case in our experience.

Clinical features of the syndrome include microcephaly, ocular hypertelorism, flat nasal bridge, upslanting palpebral fissures, bifid uvula, and cleft palate; skeletal abnormalities are frequently present, in particular radioulnar synostosis, genu valgum, pes cavum and clinodactyly, hypotonia and hyperextensible joints. Underdeveloped genitalia with classic hypergonadotropic hypogonadism are almost always present at diagnosis [61]. These patients have been reported to possess an increased risk for immunodeficiency and immune-mediated conditions, such as asthma and atopic dermatitis, possibly explained by antibody deficiency and impaired immune response to polysaccharide antigens [62].

The behavior of affected people is described as timid, shy, or friendly, with some patients showing bouts of frustration that can lead to irritability [32], while the IQs of the patients range between 20 and 60 points, significantly lower than the other HGA [57, 63].

In conclusion, HGA syndromes encompass a number of very rare conditions, once considered as variants of KS, although characterized by an earlier and more severe hypergonadotropic hypogonadism and a broad spectrum of peculiar physical malformations and neurodevelopmental and behavioral disorders. Different karyotypes are associated with peculiar phenotypes, although significant overlap is observed. Age at diagnosis ranges from infancy to adolescence, owing to evidence of facial, skeletal, and genitourinary malformations. Testosterone replacement therapy is usually necessary to start or complete pubertal development, although the appropriate timing is currently unknown. Owing to their inherent complexity, HGA syndromes benefit from a multidisciplinary team management.

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Genetic and Epigenetic Aspects of the Supernumerary X Chromosome

5

Marco Bonomi, Giovanni Goggi, and Biagio Cangiano

Few years after the first clinical report of nine men with Klinefelter syndrome (KS) [1], it was discovered that the karyotype of those subjects was characterized by an extra X chromosome (genotype XXY) instead of the usual male sex complement (genotype XY). Indeed, it is now well known that the classic form of KS, which is present in the 80–90% of cases, is defined by a 47,XXY karyotype, whereas higher grade aneuploidies (e.g., 48,XXXY or 48,XXYY), structurally abnormal X chromosome (e.g., 47,iXq,Y), or mosaicisms (e.g., 47,XXY/46,XY) explain approximately the remaining 10–20% of cases (Table 5.1).

The presence of one, or more, supernumerary X chromosome(s) comes from a sex chromosome non-disjunction occurrence, which represents a failure in chromosomes' partitioning during anaphase, giving place to cells with an aberrant number of chromosomes (i.e., karyotype 47,XXY in KS). Non-disjunction may be paternal (50% of cases), maternal (50% of cases), or post-zygotic (Figs. 5.1 and 5.2) Indeed, it happens most often during oogenesis or spermatogenesis because of an unsuccessful separation of chromosomes or chromatids during gametogenesis; however, in about 3% of cases, it may also happen during the early mitotic divisions of the zygote. While a maternal chromosome X non-disjunction may occur during both the first and the second meiotic division, a paternal supernumerary X chromosome can only be the result of a non-disjunction during the first meiotic division, since anomalies in meiosis 2 would give place to XX or XY sperm cells, leading to XXX or XYY zygotes.

The advanced maternal, and possibly paternal, age is a well-known risk factor for KS. Indeed, it has been reported that an advanced maternal age may be a risk factor for the genesis of KS: in fact, in mothers older than 40 years, the prevalence of KS

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Table 5.1 Klinefelter karyotype distribution

Karyotype	Frequency
47,XXY	80–90%
47,XXY/46,XY	6–7%
48,XXYY	~1%
48,XXXY	~2%
49,XXXXY	~1.5%

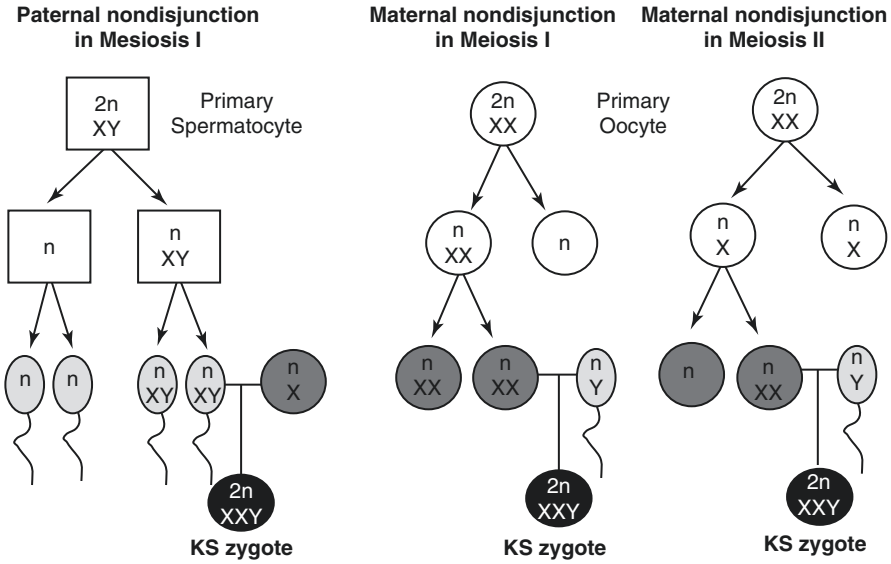

Fig. 5.1 Meiosis nondisjunction and KS aneuploidy

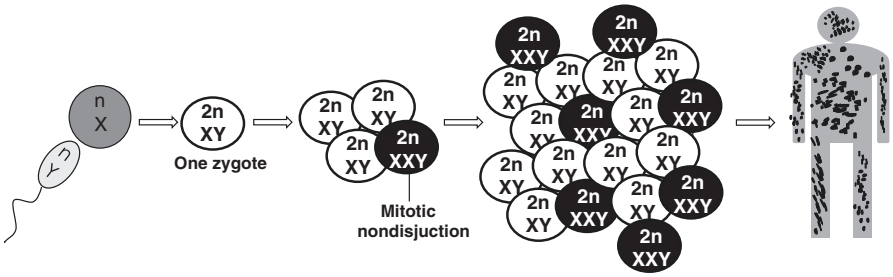

Fig. 5.2 Mitosis nondisjunction and KS aneuploidy

seems to be four times higher than in mothers younger than 24. It has also been reported that the maternal age may also have influence on a mitotic non-disjunction in the zygote: the older the mother is, the higher the chance of mitotic errors and therefore the possibility of a post-zygotic X chromosome non-disjunction as well. As mentioned above, up to 80–90% of all KS subjects present a classic 47,XXY karyotype, while the second most common form of genetic background, present in

around 6–7% of all KS subjects, is represented by the 46,XY/47,XXY mosaicism [2]. This latter may be the consequence to either a non-disjunction during the early mitotic divisions of a 46,XY fertilized egg or the loss of one X chromosome in a 47,XXY zygote due to anaphase lagging [3]. Only one study to date has compared KS subjects with a classical 47,XXY karyotype or 46,XY/47,XXY mosaicism [4]. In this study, KS men with 46,XY/47,XXY karyotype had larger testicular volume, lower levels of luteinizing hormone (LH) and estradiol, and higher mean total sperm count compared with non-mosaic KS ones (azoospermic 93.0 vs. 96.3%), and none of them reported any comorbidity. From this study, it would seem that patients with mosaicism tend to present a more favorable phenotype compared to non-mosaic KS subjects.

Despite the insights provided by numerous studies concerning the clinical consequences of KS performed so far, our knowledge of the molecular and cellular mechanisms underlying the KS pathogenesis is still limited in part due to the lack of in-depth mechanistic studies. Apart from the aneuploidy and that of the inter-individual genetic variation, several genetic mechanisms may play a role in modulating the phenotype variability observed in KS subjects. It deals with the number and the derivation (maternal or paternal) of supernumerary X chromosome/s, the dosage effect and the expression/inactivation status of the X chromosome genes, the presence of mosaicism, the activity of the genes located in the pseudoautosomal regions (PAR) of the sex chromosomes, the number of X-linked copy number variation, the epigenetic mechanism, and the possible role of aneuploidy in the tumorigenesis.

5.1 Maternal or Paternal Origin of the Suprannumerary X Chromosome

Some studies suggested that the possibility of a different parental origin of the supernumerary X chromosome may have an impact on the phenotype of KS patients. In fact, a few findings of a parental origin effect on KS phenotypic traits have been reported, including motor function and language/speech [5], autistic and schizotypal traits [6], onset of puberty [7], and the ratio of waist and height to arm span [8]. In particular, it has been reported that KS patients with a paternal extra X chromosome have a later onset and a slower puberty development [3]. However, most studies found no association at all between the parental origin of the supernumerary X chromosome and the clinical phenotype in KS patients [9–12]; therefore, evidence on this specific matter is still inconclusive.

5.2 X Chromosome Inactivation and Gene Dosage

In female somatic cells, the transcription of one of the two X chromosomes is randomly inactivated in order to guarantee a gene dosage for X-linked genes comparable to male cells. This process generates the so-called Barr body, which is the

inactivated X chromosome, whose condensed chromatin is microscopically identifiable. The responsible for the X inactivation is the product of the gene *Xist* (X-inactive-specific transcript) located on the long arm of the inactivated X chromosome. *Xist* gene mediates the silencing of the extra X chromosome in somatic human cells and its expression indicates the presence of a second and any other possible supernumerary X chromosome in the somatic cell. However, about 15% of X-linked genes in women manage to escape the X-inactivation process, especially those located prevalently on the short arm of X chromosome (Xp): nevertheless, female metabolism is able to adequately tolerate such doubled gene dosage for these "escapee" genes. A recent study by Skakkebaek et al. [13] showed that expression values of *Xist* in KS were equal to that seen in females, which is in agreement with other studies reporting that in KS men *Xist* methylation is comparable to that observed in women. Furthermore, the expression of *Xist* has been observed in blood cells of KS patients, and the Barr body was identified in both Leydig and Sertoli cells of KS patients, while these findings were not reported in 46,XY men. Altogether, these data suggest that in KS males, as in 46,XX females, one of the two X chromosomes is inactivated, thanks to the upregulation of *Xist* itself, comparable to that seen in females. However, just like in females, also in KS somatic cells a group of X-linked genes (prevalently on Xp) manage to escape the inactivation process mediated by *Xist*, giving place to a doubled gene dosage for these specific genes, which is a dosage equal to that seen in females, but unusual from what is normally seen in males. Therefore, the X-inactivation escape of these genes may be responsible for KS phenotype, since male metabolism may not be suitable to bear such a female gene dosage for these X-linked genes. The *TXLNG* and *EIF2S3* are two examples of genes located on X chromosome that have been demonstrated to escape the X-inactivation process and whose triple gene dosage may play a role on KS phenotype [13]. In fact, *TXLNG* product seems to regulate bone mass density, while the *EIF2S3* one seems to be linked to mental retardation, hypogonadism, obesity, and the regulation of lipid metabolism. Therefore, these two genes may be involved in several of the comorbidities of KS, such as osteoporosis, dyslipidemia, diabetes, obesity, cognitive disorders, hypogonadism, and azoospermia [13].

Moreover, the same authors have also revealed that several X-linked genes are differently expressed between KS and females as well as between males and females, which may suggest that the X-inactivation pattern in KS men is not completely equal to that seen in women, but may be more similar to that seen in 46,XY men [13]. This is the case of *KDM5C*, an X-inactivation escape gene, differentially expressed in KS compared to both male and female controls, that may possibly play a role in the neurocognitive dysfunction in KS subjects [13].

While male somatic cells do not undergo the X-inactivation process, in male germ cells the situation is quite different. Earlier studies already reported that male germ cells are the only ones expressing the gene *Xist*, therefore leading to the conclusion that the single X chromosome in germ cells is usually inactivated. However, later studies proved that this X inactivation does not occur completely in adult spermatogonia, since a large group of X-linked genes are still expressed in male germ

cells. Among this group, about 99 genes (10% of all X-linked genes) belong to the so-called cancer–testis antigen family and represent a smaller group of testis-specific genes, whose proper gene dosage is essential for the survival of the germ cell within the mature testis. Therefore, in germ cells of KS patients, the doubled gene dosage of these testis-specific genes (a first copy from the regular X chromosome and a second "X-inactivation-escaped" copy from the supernumerary X chromosome) may compromise the testis function and alter meiosis itself, playing a possible role in the genesis of infertility in KS patients. Among these genes, a recent study proved that the overexpression of the gene *TEX11* (*Testis-expressed 11*), normally involved in the suppression of cell proliferation, may contribute to germ cells death, making *TEX11* a potential candidate for the failure of spermatogenesis in KS.

5.3 Genetic Aspects Related to the Androgen Receptor

The role of the androgen receptor, AR, in determining differences in KS phenotype is still a topic of debate because evidence is still inconclusive about this. The N-terminal domain of the exon 1 of the *AR* gene (*AR*, mapped on Xq11.2–12) contains a sequence of CAG repeats and it is highly polymorphic: the length of the CAG repeat sequence is negatively correlated with the function of the AR, which means that the longer the receptor is, the lesser its activity and vice versa. From a theoretical point of view, with the skewed inactivation of the supernumerary X chromosome, one of the two *AR* gene alleles is randomly inactivated as well. However, different studies suggest that the choice of which X chromosome to inactivate is influenced by the length of its *AR* allele (depending on the number of CAG repeats). For example, Zitzmann et al. [14] reported a preferential inactivation of the X chromosome containing the shorter *AR* allele (therefore, with higher receptor activity), while Suzuki et al. proved the opposite [15].

Besides, some studies also showed that the different CAG repeat lengths of the expressed AR might explain some of the variabilities in KS patient's phenotype. For example, Zitzmann and colleagues reported that KS patients expressing the longer *AR* allele (with lower receptor activity) tend to present a more severe clinical phenotype compared to those expressing the shorter *AR* allele [13]. More recently, a study by Bojesen et al. demonstrated a positive correlation between CAG sequence length with final height and arm span, while a negative correlation with levels of cholesterol and hematocrit [16]. However, such correlation was found without any significant evidence of a preferential inactivation of *AR* allele depending on its length. In addition, concerning anthropometric parameters, some other studies showed a positive correlation between the *AR* length and elements such as height, arm span, arm length, and leg length [2]. However, as for other clinical elements, evidence is more inconsistent: in fact, regarding testicular volume, gynecomastia, lipid metabolism, and bone-related parameters, some studies found a negative correlation between these measurements and CAG repeat length, whereas others found no correlation at all [2]. In another study, Wikstrom et al. reported that KS patients

expressing longer *AR* present later onset and slower pubertal progression and slower testicular degeneration process, which is consistent with the finding of a positive correlation between height and arm span [7]. In addition to these findings, individual studies have also investigated and reported an inverse correlation between CAG repeat length and penile length, attained educational level, and chances of entering partnership [2]. Despite all this, however, other studies did not find any evidence at all of a link between neither the number of CAG repeats and a preferential inactivation of *AR* nor the length of *AR* and other clinical manifestation of KS. Conclusively, while CAG repeat length seems to be related to some different phenotypical elements in KS, especially anthropometric measures, the effect of *AR* length on KS phenotype remains a topic that needs further studies.

5.4 Role of the Genes Belonging to Pseudoautosomal Regions (PAR)

The pseudoautosomal regions PAR1 and PAR2 are short, homologous regions between chromosomes X and Y: they undergo crossing over during meiosis, just like two real autosomes, and their inheritance is also autosomal rather than sex linked. PAR1 is located at the terminal region of the short arm of sex chromosomes, and in this region 24 genes have been identified, being half of them with a known function; they play an important role in the pairing of sex chromosomes during meiosis, which is an essential step for spermatogenesis. PAR2 is located at the tips of the long arms of sex chromosomes, and only four genes have been identified to date within it.

All genes belonging to PAR1/2 normally escape X inactivation in females, which means that usually a double gene dosage is present for each of these genes in both males and females. In KS subjects, PAR genes escape X inactivation as well; therefore, they are expressed thrice, thus possibly influencing the final clinical phenotype.

Among all genes located within the PAR1/2, the only one that was proved to be able to influence the KS phenotype is *SHOX* (short-stature-homeobox-containing gene on chromosome X), which belong to PAR1. Indeed, in KS subjects, tall stature and long extremities are evident since the early childhood, long before pubertal development, despite normal levels of IGF1 and IGFBP3: hence, this suggests that hypogonadism alone cannot explain completely KS phenotype, and a plausible explanation for this characteristic may be an overexpression of genes related to growth, such as *SHOX*, which is in fact the only gene on the sex chromosomes that has been convincingly connected to the KS phenotype.

Another example of pseudoautosomal gene significantly expressed (from three loci) in KS is *SLC25A6*: this gene is involved in calcium signaling pathway and metabolism and seems to be correlated with short QTc, which could explain part of the molecular mechanism behind short QTc in KS [13].

5.5 X-Linked Copy Number Variations (CNVs) in the Klinefelter Syndrome

Another possible mechanism that plays a potential role in the clinical KS phenotype was recently identified in the X-linked CNVs (copy number variations). Indeed, it was recently demonstrated that KS is associated with high recurrence of CNVs on the X chromosome. Authors reported that KS patients present more frequently (41.5 vs. 28.6% of females and 18.6% of males) and more numerous X-linked CNVs compared to female and male controls [17]. Almost all X-linked CNVs in KS subjects are duplications and map within regions encompassing genes. Moreover, it was also demonstrated that most of these CNVs affect genes that usually escape the X-inactivation process in regions of X–Y homology, especially those located in PAR1 and Xq21.31. Therefore, a duplication of one of these genes in KS raises the number of its copies from 3 to 4, further raising the already increased gene dosage of these PAR genes and possibly playing a role in the clinical phenotype of KS.

5.6 Involvement of Epigenetic Mechanisms

It has been proposed in many studies that differences in gene expression (and not in their sequence) due to epigenetic modifications of DNA might be considered as another important mechanism involved in the variability of the KS phenotype. Indeed, epigenetics is defined as "the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence". Epigenetics does not involve DNA sequences of genes, but instead it studies their different expressions due to histone variants, post-translational modifications of amino acids on the amino-terminal tail of histones (such as acetylation and methylation), and covalent modifications of DNA bases [18].

A recent study by Belling et al. [19] reported that KS patients have more than 300 differentially expressed genes compared to male controls. Interestingly, most of them were not X encoded but located on autosomal chromosomes. Thus, it appears that having an extra X chromosome can influence gene expression across all the genome. In addition, their analysis also indicated that the dysregulation in genes expression involved the immune system and the energy balance, both known to be affected by KS. In particular, the dysregulation of *IL-4* gene (normally playing an essential role in inducing adaptive immune response) is possibly involved in infections and autoimmune diseases, which KS patients have increased risk of (including autoimmune diabetes mellitus) [19].

Another study by Skakkebaek et al. [13] also reported that methylation of CpG sites in KS genome is different compared to controls: in particular, a global preferential hypermethylation of the genome seems to be present, in agreement with other earlier studies. In particular, consistent with the previous report [19], the predominant hypermethylation is diametrically opposite to what has been observed in Turner

syndrome (45,X): this could imply that a gain or loss of an X chromosome in humans causes epigenetic alterations, which may influence the phenotype of patients with sex chromosome aneuploidies through an effect on transcriptional and translational regulation. Moreover, the authors also identified specific differently methylated genes in their KS patients, four autosomal and one X linked: *SPEG*, *G3BP1*, *NSD1*, *ZNF497*, and *SHROOM2*. Based on the known functional roles of these genes, it has been proposed that their methylation changes could be implicated in the phenotype of KS, such as delayed motor development, decreased muscle strength, cardiac abnormalities, cognitive deficits, and endothelial dysfunction. Moreover, their data also suggest that KS may be also associated with a deregulation in Wnt signaling, which is an alteration already known to influence diseases such as cancer, embryonic development and malformation, bone density alterations, and diabetes. Besides, Wnt signaling is also involved in synaptic activity and plasticity and may be crucial for normal learning and memory. Thus, deregulated Wnt signaling in KS may be involved in comorbidities, such as osteoporosis, diabetes, vascular comorbidities, congenital malformations, and cognitive deficits in learning and memory [13]. Skakkebaek et al. also showed a downregulation of the gene *AMOT* (X linked), which is normally expressed in endothelial cells of capillaries, compared to both female and male controls. It is possible that this different expression in KS might explain at least part of the endothelial dysfunction typical of KS, and therefore it might be involved in the pathogenesis underlying some of KS comorbidities, such as hypertension, diabetes, atherosclerosis, and erectile disfunction [13]. Finally, several non-coding genes were found to be differentially expressed in KS compared to both male and female controls, many of which with unknown functions: what their role may be in determining the phenotype of KS is yet to be uncovered. However, some of them are peculiarly located close to the X chromosome inactivation center or to some escape genes (e.g., *KDM5C*, *ZFX*, *EIF2S3*), suggesting that such non-coding genes may be involved in the regulation of the X-inactivation process and the expression of escape genes [13].

In 2018, a study by Salemi et al. [20] reported a significant downregulation of *MT-ND6* (mitochondrially encoded NADH:ubiquinone oxidoreductase core subunit 6) gene in KS patients DNA compared to male controls. *MT-ND6* is a mitochondrial subunit belonging to Complex I, encoded by mitochondrial DNA, thus involved in the respiratory chain. It has been proposed that mutations in mitochondrial subunits (such as *MT-ND6*) may lead to defects in the assembling of Complex I, resulting in Complex I deficiency, which is known to be responsible for many pathological conditions. Therefore, the authors suggest that the downregulation of *MT-DN6* may lead to decreased Complex I levels, which would induce cell apoptosis, thus playing a role in the pathogenesis of KS phenotype.

Lastly, another study by Cimino et al. managed to identify two strongly downregulated miRNAs in KS compared to male controls (*MIR3648* and *MIR3687*), which seem to play a role in breast cancer, hemopoietic abnormalities, immune defects, and adipocyte differentiation and maturation. These data might explain

some of the phenotypical elements in KS, such as immune and metabolic disorders and the higher risk of breast cancer compared to 46,XY men [21].

Altogether these data are indicating a role of the epigenetic mechanisms in the clinical phenotype of the KS. Nonetheless, it has been reported that some studies failed to identify the correlation between the gene altered methylation and the relative gene expression [13]. The missing correlation with the gene expression indicates that the link between DNA methylation and gene expression is more complex and the biological function of the altered methylome seen in KS needs to be further investigated.

5.7 Aneuploidy and Cancer

Recent studies suggest that aneuploidy might have a role in tumorigenesis in general; therefore, it is interesting to speculate that the presence of a supernumerary X chromosome might at least partially explain the reason of the increased occurrence of cancer in KS patients. However, evidence about this matter is not univocal, since it has been shown that aneuploidies can both promote and suppress tumorigenesis.

Several studies found positive correlations between the degree of aneuploidy, proliferation, and cell cycle transcriptional signatures that are believed to be indicative of promoting tumorigenesis [22–24]. Therefore, from these analyses, it would seem that aneuploidy confers a selective advantage and increases the tumorigenic behavior of human cancer cells. On the other hand, some other studies revealed that single-chromosome gains lead to slower proliferation and various detrimental metabolic and physiological consequences in triploid cells, which show a reduced tumorigenicity compared to their diploid counterparts [25, 26]. Therefore, from this evidence it would seem that aneuploidy confers a fitness penalty and suppresses rather than promotes tumorigenesis. Hence, the role of aneuploidy in cancer is not univocal, since it has not been proved, under specific circumstances, to confer a fitness advantage; on the contrary, it has been proved to be detrimental to cell proliferation and survival. Tumor stage, cell type, genetic makeup, tumor microenvironment, and immune system interactions all determine the circumstances under which aneuploidy can drive tumorigenesis. For example, in many cancers, aneuploidy increases with tumor progression. However, in some tumors (such as breast or lung cancer), aneuploidy has been observed at the stage of carcinoma in situ, suggesting that it may confer a selective advantage early in tumorigenesis. As for the cell type, it has been shown that the same chromosome is commonly gained in one tumor type, but frequently lost in another, demonstrating that no single chromosome gain or loss universally promotes tumorigenesis [27].

Therefore, according to all the evidence, we believe that the extra X chromosome in KS patients is not necessarily implicated in tumorigenesis, especially if the circumstances required to increase it are lacking.

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Prenatal Counselling and Management in the Early Neonatal Period

6

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6.1 Prenatal Diagnosis and Screening of Chromosome Variations

Since 1970 the detection of chromosome aneuploidies has become routine and is increasingly used as a worldwide strategy in obstetric practice. The prenatal tests enable prospective parents to get essential information about foetal health and to make informed decisions about the pregnancy.

Prenatal genetic diagnosis (PD) currently still requires the collection of a sample of foetal cells by either aspirating chorionic villi by a transabdominal approach under ultrasonographic guidance at 10–14 weeks of gestation or withdrawing amniotic fluid and collecting and culturing exfoliated foetal cells (amniocentesis) at around 15 weeks of gestation (Fig. 6.1). These procedures generally carry very low rates of post-procedure miscarriage, less than 1/300, according to the Danish Fetal Medicine Study Group [1]. The major advantage of chorionic villus sampling (CVS) over

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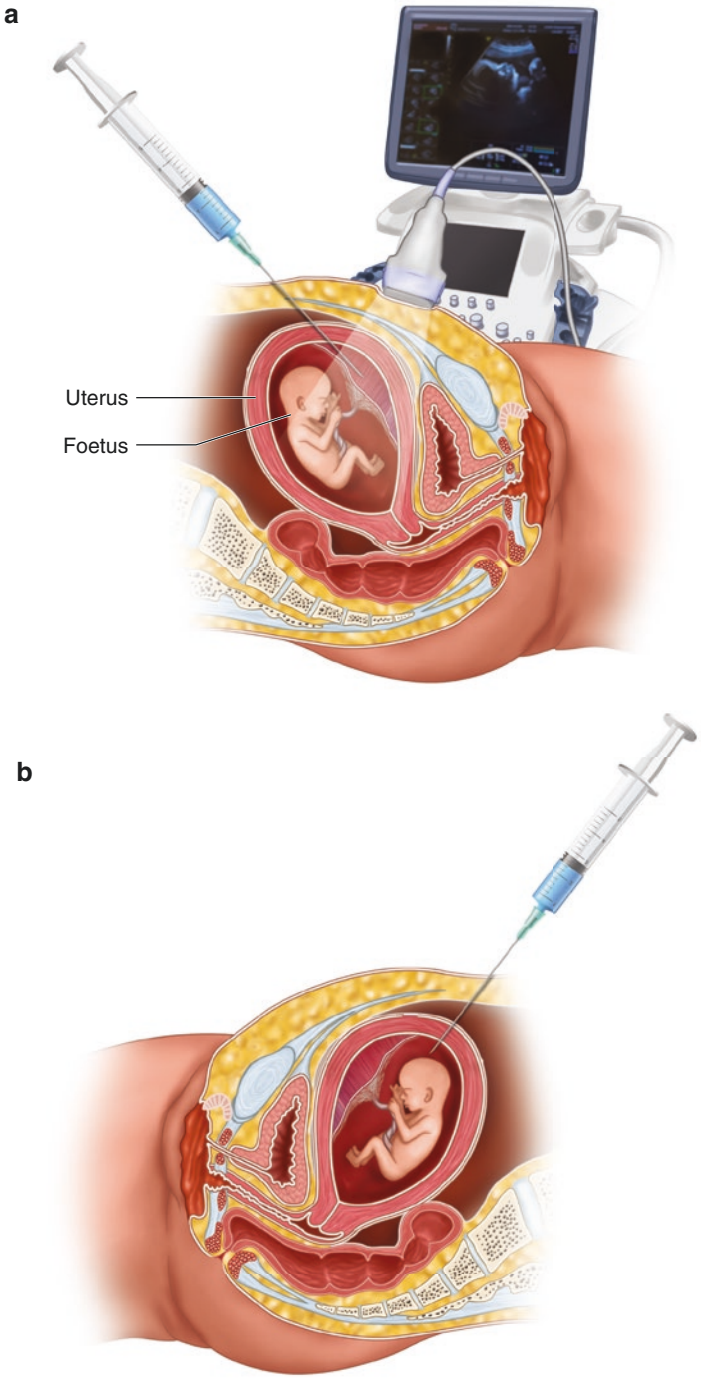

Fig. 6.1 (**a**) Schematic representation of chorionic villus sampling. (**b**) Schematic representation of amniotic fluid sampling

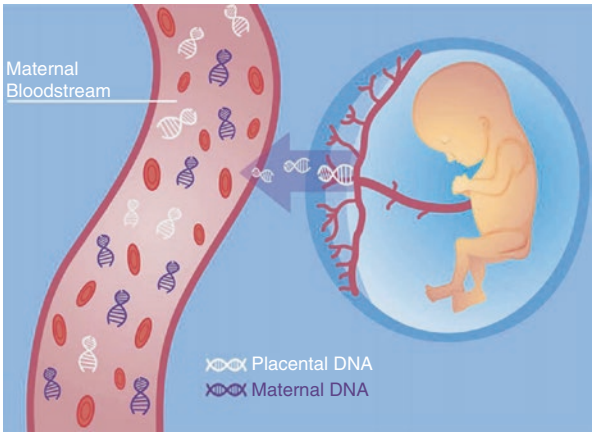

Fig. 6.2 Schematic representation of the placenta contribution of cfDNA (fetal fraction) circulating in the maternal plasma

amniocentesis is that it can be performed at a much earlier stage of pregnancy and entails a minor physical and probably smaller emotional risk to the woman who receives a positive result and requests a termination of pregnancy (TOP). However, compared with amniocentesis, when CVS is used for a foetal karyotype, there is a greater likelihood of needing a repeat test because of unclear results, mainly mosaicisms, which are detected in 1 case out of 50 [2, 3].

More recently, that is in the last decade, clinicians have witnessed an incredible change of paradigm with the shift from traditional invasive prenatal diagnosis toward non-invasive prenatal screening (NIPS), that is, Combined Test (CT) and cell free DNA (cfDNA) or Non-invasive Prenatal Screening (NIPS). Since its introduction in 2011, prenatal screening using cell-free DNA extracted from maternal plasma has expanded at a steady rate, both in the USA and in Europe [4]. cfDNA provides for the analysis of genotypic marker DNA directly from the placenta which delivers a considerable amount of foetal DNA (foetal fraction) into the maternal circulation (Fig. 6.2). Cell-free foetal DNA analysis present in maternal blood can be studied either through a quantitative 'counting' method that uses targeted parallel sequencing or by identifying maternal and foetal allele distributions using single-nucleotide polymorphisms (SNPs) [5]. Initially, cfDNA screening was validated for detecting foetuses with possible trisomy 21 in pregnancies at high risk for aneuploidy. Soon after in 2012, screening for trisomies 13 and 18 became available. The biggest impact of this technology is the fact that cfDNA screens can be performed very easily and can also identify the sex of the foetus as well as detecting sex chromosome aneuploidies (SCAs), including, among others, Klinefelter syndrome.

Once a chromosome variation has been suspected by the test, it is very important to discuss with the couple the option of an invasive procedure to obtain foetal cells for traditional testing. This may uncover false-positive screen results as well as discrepancies among the suspected variation and the real foetal karyotype, sometimes with different clinical prognosis [6–8]. In our experience, we have had five pregnancies in which a high risk for 47,XXY foetal constitution after cfDNA test has been turned into a 48,XXYY foetal karyotype on amniotic fluid cells.

6.2 Need for Counselling and Good Communication

Women in the general population do not have opportunities for structured genetic counselling before undergoing prenatal diagnosis (PD) to learn about the importance of prenatal testing and to consider what difficulties and what difficult choices may arise from the tests. Moreover, they are very often unaware of the sex SCAs which are among the most common disorders encountered. The SCAs constitute about 25% of all chromosomal abnormalities detected after amniocentesis with an overall incidence of 1 in 300–340 [9, 10], their specific prevalence being 1/660 for 47,XXY, 1/700 for 47,XXX and 1/1000 for 47,XYY [11]. These anomalies are detected by all testing modalities, including traditional cytogenetic, quantitative fluorescent-polymerase chain reaction (QF-PCR) rapid aneuploidies test, array comparative genomic hybridization (CGH) and non-invasive prenatal screening (NIPS).

Concerns have been raised about the increased demand on the part of the women and on indiscriminate offers of testing by commercial interests without appropriate risk assessment and, most importantly, without proper pre-test counselling.

For this reason the professionals who assist the women/couples who have opted for prenatal testing should give careful consideration of how these possible diagnoses should be communicated to the potential parents.

The first communication is crucial because it could affect how information presented later is interpreted by parents [12, 13]. Moreover, even the decision of continuing the pregnancy could be affected by how the diagnosis is first communicated. For example, the parents in the Abramsky et al. study reported that some disclosures were done by non-expert people, in many cases midwives or obstetricians not specifically trained to communicate a diagnosis, and that the information provided at the first communication was often misleading and not up-to-date [12]. Therefore, the authors concluded that a protocol for communicating results to parents should be implemented in all the units providing prenatal testing services.

6.3 Pre- and Post-Test Session

It is our belief that each institution offering PD or NIPS should design a protocol to assist the prospective parents in dealing with unexpected detection of SCA. For instance, we suggest that a more detailed counselling session concerning sex chromosome trisomies should be conducted before the prenatal testing, to increase the positive adjustment of parents involved in prenatal diagnosis [14]. During the pre-test counselling, a clinician should carefully structure the manner in which the diagnosis will eventually be communicated allowing time and space for extensive counselling as well as activation of the multidisciplinary team.

Once the diagnosis is made, regardless the technology applied, the first step is to provide accurate information about the chromosomal variation identified, its origin and the accuracy of the laboratory report. Then, clinical consequences of the conditions, including phenotypic features, management and/or treatment, quality of life, life expectancy and social aspects, including available support and relevant research

developments, should be presented. This should be supplemented, if possible, by written information and genetic counselling. Also, referral to other expert health professionals, if appropriate, would promote a multidisciplinary approach.

Indeed, couples often request the presence of a paediatric endocrinologist during the counselling. Although counselling tends to focus on long-term endocrinological implications of this diagnosis and on the early management of symptoms, the role of the endocrinologist is mainly that of support, since hormonal and fertility issues do not represent a treatable problem at this time. Thus, early on the endocrinologist should be there primarily to reassure the parents about the physiological growth during the first years of life and to guide the referral to a general practitioner for periodic follow-up. In the absence of specific problems (such as growth defects, cryptorchidism, micropenis and hypospadias), it is reasonable to recommend a first endocrinological evaluation around the pre-pubertal period (i.e. at about 8 years). This evaluation represents the most appropriate time to focus on hormonal and fertility issues, taking into consideration that, in particular for fertility preservation, no specific guidelines are yet available [15–17].

After disclosure of the test results and the first session with the clinical geneticist and endocrinologist, the second focus should be on the emotional impact on the counselee and other involved family members. Depending on the resources available, follow-up contacts with the genetic unit should be offered, as well as consultation with a psychologist. Facing a prenatal diagnosis of an altered genetic condition may well represent a real nightmare for the future mother and father and might completely upset their relationship and their well-being, as well as their sense of self including their perceived capacity to cope with whatever may follow. The uncanny aspects of the unexpected may evolve into distortion thoughts and unreal fantasies.

It has to be recognized that parents asking prenatal diagnosis usually want to be reassured and are unprepared for a ‘non-normal’ verdict. Therefore, the couple has to be trained in the preconception phase or at least in the pre-test session to anticipate and to try to develop a strategy to be able to deal even with abnormal results, a regular unavoidable outcome of prenatal investigation.

Since prospective parents have no knowledge of clinical consequences of SCA, the phenotype of their child needs to be cautiously and gradually explained emphasizing that prenatal testing is not a clinical diagnosis and certainly does not describe ‘the child’ [18].

A wide area of uncertainty exists on how, when and who can help in differentiating for the parents between a genetic constitution and the real condition of their child. The initial shock after the diagnosis of foetal SCA may evolve into trauma if the communication is not sufficiently neutral, clear and gradual: that is why, a multidisciplinary team has to be in place for a couple to make the most reasoned choice of which they are going to face. Maternal bonding may be affected and mother may continue the pregnancy but in a particular state of mind very close to reduced consciousness, where the words spoken by the professionals during the prenatal counselling session interferes with a healthy understanding and the development of more positive beliefs [19].

Thus, the communication process has a crucial role, being a ‘cornerstone’ in facilitating a better comprehension and preventing the development of a state of mourning (i.e. the grieving process).

The possibility of contacting patient/lay support organizations should also be offered. A written summary of the test results and issues discussed during the counselling should be, as a rule, given to the counselee(s). A strategy to inform relatives should be discussed with the counselee (or, if necessary, a decision to discuss this further, after enough time has elapsed to allow their emotions to settle down).

At all times women and their partners should encounter highly trained professionals able to support them and understand their needs. Finally, we believe that is respectful of the prospective parents to explain the options that will be available after the results are known, such as continuing with the pregnancy, or a termination of pregnancy (TOP) as a rare but still requested procedure. Parents need to be always allowed sufficient time to think through their decision and made aware of the legislation concerning TOP in Italy.

In the recent years telemedicine is gaining more and more attention as a resource for clinicians to provide immediate and exhaustive genetic counselling even in remote area, after prenatal diagnosis and testing in prenatal setting [20]. The Italian Ministry of Health has issued the first guidelines for the correct use of this resource in 2015.

6.4 Internet Challenge

The power of the Internet cannot be underestimated. It is inevitable that parents, once the diagnosis has been disclosed, will try to find answers and insights on websites and/or blogs or forums. This can deeply affect their emotionality and compromise the ability of the expert professional team to communicate appropriate and helpful information. For this reason, it is crucial that the communication protocol provides for a real agreement among all parties not to deliver the result of the test by phone or e-mail before a formal counselling. Consulting the Internet after a complete explanation of the possible clinical outcomes will be much more useful and responsive to the needs of the couple.

In addition to this, the possibility of informing future parents of the resources represented by National Lay Associations, which will be available to them in their particular localities, favours the contact and sharing of concerns and fears with those who have already had direct experience with 47,XXY.

6.5 Towards the Birth of the Child

At the end of the decision-making process about the continuance of the pregnancy, which requires different lengths of time for every couple and family, the follow-up of the pregnancy should be discussed and scheduled. This will increase the chance of benefits from prenatal diagnosis of 47,XXY [21].

A complete scan should always be offered after parents communicate the decision of carrying the foetus to term. Congenital anomalies are as frequent as in any other population, but the reassurance given by a normal scan will likely be very important to the mother.

Meeting the neonatologist will also be useful since the birth of the child is the first and most important step toward normalization of their emotional status.

The newborn with Klinefelter syndrome does not show any particular symptoms, so that neonatal care is completely unchanged from the usual. This is the case if both clinical checks and diagnostic tests are considered, but the scenario changes if we keep in mind the period of pregnancy in which the unexpected diagnosis was made. In this crucial period, some questions must be taken into account and we must consider if the parents had previously been seen by an experienced person in this field and what kinds of information was conveyed to the parents and if so, what had they actually understood by it. Gynaecologists, obstetricians and nurses, even if not experts in the follow-up of these children and without the intervention of geneticists or psychologists, can provide a useful professional support to these families, first of all by helping mom and dad in all common steps that parents have to learn in the care of their child, such as breast-feeding and hygiene.

It is important that this assistance is balanced, thus appreciating what they are doing and supporting but not replacing them.

In this context, to ensure every possible contribution to the well-being of the newborn and his parents, it is essential that unnecessary procedures, delays or misunderstandings related to poor coordination between healthcare professionals should be scrupulously avoided. In any event, a repeated neonatal karyotype is warranted to provide the parents a laboratory report with the name of the child.

Informing the parents about the various interventions must be as uniform as possible in terms of content and form, so that they feel guided and accompanied but not coerced and abandoned. Although all these measures should be guaranteed for all families, stressing these issues is of particular importance in the context of SCAs.

And what about peculiar aspects of this specific condition? There is something ‘more’ that should be done? Indeed, in every couple a newborn brings some kind of novelty, and a new balance between the parents needs must be attained. This is especially so when the novelty of a newborn child is accompanied by something different, somehow unexpected and unsettling. That is why the neonatologist, as well as any other member of the multidisciplinary medical team, should have a basic understanding of this condition and be aware of the main steps of follow-up and of the medical network that will soon take care of the family. Thus, the neonatologist has a relevant role, not only because she or he is the first professional figure that parents meet after the birth, but also because she or he acts in the most precarious period, in which the aforementioned balance has yet to be established. Hence, it is of particular importance to verify that information given prenatally has been well elaborated and internalized, to emphasize what is already understood and to clarify aspects needing further explanation. Even for caregivers, the balance between saying and doing ‘more or less’ to the family or for the baby can be challenging. The challenge is however worthy, since the care of the neonatologist can become for

parents the most tangible sign of the worth of their child, thus helping them in that crucial moment and in the following small daily steps of their lives.

As mentioned earlier, in the neonatal period, as well as in early infancy there are no pathognomonic signs or symptoms of the condition, thus a specific endocrinological intervention is not required. Genital anomalies (micropenis, undescended testis, bifid scrotum and hypospadia) are not commonly observed in Klinefelter syndrome and whether they are due to the effects of supernumerary X chromosome/s or of androgen deficiency during foetal life remains to be determined [22]. However, it is important to acknowledge the association, in particular for cryptorchidism and hypospadia, and to correctly refer the patient to a specialist for appropriate therapy [23, 24].

At our institution we create a bridge between the paediatrician and the neuropsychiatric service where a specific protocol has been activated to help parents and their child in the adjustment, acceptance and understanding of the characteristics of this condition during childhood and long after [25–27] (see Chap. 8).

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Early Developmental Pathways and Communication Good Practices

7

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7.1 Introduction

The aim of this chapter is to describe the early developmental pathways of patients with Klinefelter syndrome (KS) and communication good practices, according to the studies of the literature. Behavioural phenotype of this genetic condition is wide and heterogenous for both clinical and neuropsychiatric aspects. The spread of prenatal diagnosis demonstrated that the outcomes are much better than those described in the original literature.

Different aspects of early development will be described in this chapter, to resume the recent literature from a clinical point of view and to make recommendations to define specific follow-up in accordance with the rehabilitative windows.

7.2 Motor Development and Neurological Aspects in KS

Children with KS may show variable developmental profiles. However, there is a risk for mild or moderate delay in motor skills for which monitoring is important to intervene if necessary.

Literature shows an increased risk for motor impairment, which is present in approximately 50% of boys with KS, and this is often associated with hypotonia [1–3]. The average age of developmental milestones is about 2 or 3 months later

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than typical XY peers with a mean age of walking of 14.3 months (typical range 10–17 months). Nevertheless, in 25–50% of cases, development may progress typically without apparent need for intervention. Other common features such as mild hypermobility, pes planus with ankle pronation and genu valgum can further affect motor development and must be monitored [4]. Furthermore, coordination with specific impairment in praxis, motor planning, manipulation skills, bilateral integration, execution in motor and graphomotor skills are common areas of weakness [1, 2]. Dyspraxia is often present in individuals with KS [2, 5]. Tremor has also been reported as upper extremity intention or essential tremor [6]. Finally, several case reports and retrospective studies have associated KS with an increased risk of epilepsy [7, 8].

All these weak points can affect the ability to perform adaptive skills that are important in the domestic and academic settings such as handwriting, dressing and tying shoes and may impact other areas like the ability to participate in play-games and sports activities which have important psychosocial and adaptive implications.

Mechanisms underlying motor delay and clumsiness have not yet been outlined. Nevertheless, results according to which motor impairments seem to be attenuated in boys undergoing testosterone treatment suggest that some aspects of motor function might be directly related to androgen effects [4].

Several recent studies suggest that X chromosome may significantly impact brain morphology and many characteristics (motor, social and communicative) of KS behavioural phenotype may be rooted in neural anatomy. Many structural imaging studies have signed significant neuroanatomical differences associated with KS: They report reduced total brain volume, enlarged ventricles as well as smaller caudate, cerebellar, temporal and frontal lobe [9–11]. Furthermore, boys with 47 XXY show significant grey matter volume reduction in left and right insular cortex as well as the left orbitofrontal cortex (OFC), bilateral amygdala and bilateral hippocampi. OFC is associated with the evaluation of sensory stimuli, social decision making and the representation of facial expression and identity, while the insular cortex is involved in recognition of responses of emotional stimuli. Furthermore, amygdala reductions may be related to atypical temperament, passivity and reduced sexual desire associated with KS. All these neuroanatomic features may explain that individuals with 47 XXY show problems in identification and verbalisation of emotional states, are more easily emotionally aroused and are significantly more influenced by their emotional states when making a decision [12]. In addition, other studies showed an increase in grey matter in the parietal and occipital cortex, including sensorimotor areas; this increase in sensorimotor and occipital regions in KS could be due to a reduced synaptic maturation or pruning and these aspects may reduce synaptic efficiency and determinate sensorimotor deficits typical of these individuals [9].

Recommendations for clinicians:

1. Early monitoring of motor skills (gross motor ability, praxis, motor planning, manipulation skills, graphomotor skills) is important to intervene if necessary.

2. To improve adaptive functioning and children self-esteem, an early intervention targeting motor skills is recommended even when only mild deficits exist.
3. Orthotics are often helpful for pes planus to support motor development and prevent lower extremity pain.

Recommendations for parents:

1. Encourage and sustain adaptive skills such as handwriting, dressing and tying shoes, in order to sustain self-esteem and autonomy.

7.3 Communication and Language

Many studies reported the presence of language impairments in both children and adolescents with KS [13, 14]. Language delays and impairments could be considered one of the most distinctive traits in the neuropsychological functioning of individuals with this genetic condition, considering that linguistic competence is frequently an area of weakness relative to overall cognitive skills [15].

Delayed language milestones can be noted in early childhood in both phonological [16] and vocabulary development [17]. The first words usually appear between 18 and 24 months rather than around 12 months as in the general population [18], and the delay in vocabulary development usually persists in subsequent years. At 24 months, many children with KS are late-talking children, who are children with a vocabulary size lower than the fifth percentile [19]. However, it must be noted that although a small vocabulary size is a risk factor for subsequent language development, this fact does not imply that they would necessarily develop a language disorder.

Children with KS could compensate for their vocabulary difficulties using communicative gestures (e.g. pointing and conventional gestures) [19]. The use of such compensation strategies suggests that the main problem of these children could be a difficulty in verbal production rather than a general communicative deficit.

Language problems, for instance, in understanding complex grammatical constructions, syntactic productions and word retrieval abilities usually persist during childhood and adolescence. Pragmatics could be an area of particular impairment in this population [14] and could be related to the difficulties in socialisation frequently identified in children and adolescents with KS, and described in the following paragraphs.

Careful monitoring of first communicative skills could favour early identification of possible language impairments, and thus early intervention. Both academic and social skills may benefit from the intervention in speech and language abilities.

Recommendations for clinicians:

1. During prenatal counselling, giving parents information about the higher risk to develop language impairments could lead to better monitoring of the children's communicative skills. The awareness of this risk could help the parents in providing additional scaffolding to support their children's additional needs [20].

2. Despite a higher proportion of language impairments in individuals with KS rather than in the general population, the children with KS who show speech and language impairments usually have a developmental profile similar to that found in children with developmental language disorder (DLD) [13]. Therefore, if needed, a typical speech and language intervention, usually implemented for children with DLD, should be used with children with KS.

Recommendations for parents:

1. Careful monitoring of children's early communicative skills (e.g. use of gestures, vocalisations) could favour early identification of possible language impairments.
2. It is important to stimulate children's language development by frequent one-to-one interactions with him and by shared books reading activities since the first months of life.

7.4 Executive Function and Learning Disabilities

The neurocognitive phenotype in KS is associated with several deficits, although it displays a high variability. The general intellectual ability in KS varies from average to below average [21]. Many individuals with KS will not be significantly impacted by cognitive concerns and will achieve success in academic, personal, and career endeavours. However, statistically significant discrepancies have been shown relative to the general population and biological sibling controls. In most studies, verbal IQ scores are found to be lower than performance/nonverbal IQ scores [4, 21].

The clinical aspects of KS tend to be language-related disorders [22]. In fact, as language skills advance and become more complex, higher-level language deficits are common, including a poor grasp of verbal concepts, verbal processing difficulties, slow verbal processing speed, decreased verbal fluency, word retrieval problems, social communication difficulties and difficulty with open-ended narrative construction [4]. Boys with KS are at higher risk than the general population for language-based learning disabilities, including reading and spelling [4, 21]. Occurrence ranges between 50 and 80%, and a family history of learning disabilities increases this risk [23]. More broadly, approximately 80% of boys with KS require some form of specialised support in school for language-based learning or reading concerns: this is typically provided in the form of an Individualized Education Plan (IEP) and it is strongly recommended that providers take an assertive stance about advocating for intervention and educational supports [4].

The majority of individuals with KS also display impairments in Executive Functions (EF): having an extra X chromosome is associated with abnormal structure and function of brain areas in the frontal lobe, which is crucially involved in EF [24]. EF are considered essential for flexible adaptive functioning in complex situations and refers to cognitive control processes involved in goal-directed behaviour, problem-solving and processing thoughts in a fluid and flexible way. EF dysfunctions may be characteristic for individuals with KS, including working memory,

cognitive flexibility, task initiation, fluency and inhibition [4, 24]. There is also an increased risk for attentional problems: distractibility and inattentive symptoms are more common than hyperactivity and impulsivity [4], prevalently between 36 and 63% of cases [25]. Weak inhibitory control can lead to impulsive behaviours and acting without taking into account the associated risks and consequences of the action; children with more inhibition difficulties had higher levels of thought problems, aggression and rule-breaking behaviour [24, 26].

Recommendations for clinicians:

1. Referral for comprehensive developmental assessments for all children using standardised measures from preschool education: Early interventions including developmental, speech, occupational, physical or behavioural therapies are much better than tardive.
2. Special education supports (IEP) as needed. Consideration of additional academic supports, tutoring, options for schools/educational settings. Involvement of school team.
3. Multi-professional assessment (psychological, neuropsychological and neuropsychiatric) for cognitive functioning, learning disabilities (reading and mathematics), EF and attention beginning at 7–8 years of age, and at key times during education and transitions.

Recommendations for parents:

1. Education of parents/caretakers about EF and manifestations of symptoms of EF deficits. Supporting that the disclosure process includes discussing the assessment gradually, honestly, and simply with age-appropriate terms and a positive attitude also with child.
2. Implementation of educational strategies and supports for EF at school and home.
3. Consideration of the role of speech, EF and learning difficulties in behaviour/frustration.

7.5 Behaviour and Emotional–Relational Aspects

Behavioural characteristics of children with KS have been variously studied in the literature to define the behavioural phenotype, which is very wide and heterogeneous. Knowing the changes in children's development is important to describe behavioural characteristics, especially in the first years of life. In particular, impaired motor skills and language delays, in both receptive and expressive skills, could penalise attachment to caregivers, adaptive and social functioning. Language difficulties and communicative impairment make it difficult for children to talk about themselves and express their needs. Children's ability to emphatically understand and regulate emotion is really important. A specific difficulty in coding and express emotions is related to children's ability to emphatically understand and express them [27]. In scholastic or social situations, a significant level of social anxiety could emerge, due to the difficulty with word retrieval that penalises social

participation and adaptative functioning [4]. The child could react with oppositional and aggressive behaviours as compensation for the perceived frustration. In some cases, are also reported thought disorders [24].

Behavioural characteristics in these children, associated with psychological and developmental risks, are often reported in review studies. Low self-esteem and socio-emotional immaturity could penalise interaction with peers and increase levels of concern for the relationship.

Difficulties in socialisation have also been associated with the presence of behaviours related to neurodevelopmental disorders, in particular to autistic spectrum disorders (ASD) [4]. In two studies, respectively, conducted on cohorts of 19 and 34 paediatric patients [28, 29], the percentage of children with KS, diagnosed with ASD, was approximately 11 and 12%. Inclusion criteria, according to *Diagnostic and Statistical Manual of Mental Disorders Fourth Edition* (*DSM -IV*) or *DSM-5*, are one of the main problems for an accurate diagnosis of ASD in KS. According to the *DSM-5* diagnostic criteria and by the use of an assessment with specific tools (*Autism Diagnostic Observation Schedule* - ADOS 2, *Autism Diagnostic Interview* - ADI), the percentage of diagnosed patients, in cohorts of children, is around 10% higher than using the *DSM-IV* inclusion criteria. Poor eye contact, language delay, hypersensitivity to sensorial stimuli and restricted interests are behaviours that can easily overlap the functioning of children with KS with that of children with ASD. In several studies, children with KS have shown less attention to social events, lower competence for emotional tuning and interpretation of other's facial expressions.

Recommendations for clinicians:

1. Multi-professional approach and up-to-date diagnostic tool.
2. Use of standardised and evidence-based assessment to evaluate behaviour and adaptive functioning in children with KS and their families [28].
3. Define an evaluation made up of direct and indirect tools.
4. Encourage attention in children and their families to manage frustration and behavioural problems with strengths.

7.6 How and When Communicating to the Child

Diagnostic communication to children or young people with genetic conditions is a topic hardly addressed in the scientific literature. Nevertheless, common concerns and issues may be identified in the paths of these families [30].

Specifically, various works highlight how parents are especially concerned about not knowing how to choose the most appropriate moment to convey the communication and that this may upset the child, or that the knowledge of the diagnosis by the figures involved in the family life context may be a source of prejudice [30–32]. Furthermore, limited support from specialists emerged in the disclosure process by parents, as they actively intervene to support families in a percentage that varies over time from 10% [30] to 42% of the cases [33]. Studies on diagnostic communication to young patients with aneuploidy of sex chromosomes, confirm the aforementioned considerations [33].

The main concerns of these parents are related to the idea that the child is not ready and cannot understand information regarding his genetic condition [34] or that diagnostic communication gives rise to a perception of inadequacy and diversity that could lead to feelings of sadness and low self-esteem.

Patients do not confirm these concerns. Patients report, instead, receiving the diagnostic communication with neutral feelings in the 50% of the cases, with relief and positively in 25% and with negative feelings and concerns in the remaining 25% [33]. Patients report, in most cases, that they would have liked to be informed early by parents, with clear and non-denying words, starting from what they perceived as disturbing to their life experience. Parents also declare their concern that the disclosure of information regarding the genetic condition in the child's living environment may generate prejudices and differentiated treatments [34]. For this reason, parents frequently ask the child not to share his diagnosis outside the family [33].

In light of these considerations, although every intervention must always be thought of taking into account the subjectivity of the individual child and his family, it may be useful to outline some recommendations, for specialists and families, on how and when to convey the genetic diagnosis to the young patient with KS, similar to what Tremblay did [35].

Recommendations for clinicians:

1. Provide detailed and in-depth information regarding the genetic condition, because a competent parent is able to convey a simpler and more effective communication to their child [30].
2. Provide selected information material: scientific articles, brochures and illustrated books for children [36, 37].
3. Encourage parents to inform the child as early as possible, as the secret is a source of stress and anxiety for the entire family system.
4. Propose ad hoc paths, such as the activation of groups of parents or support session with the parents, to support them in the delicate moment of communication to the child.
5. Not to replace the parent at the time of communication, whenever possible, and be available to meet the child at a later time, if necessary.
6. Advise completing the path of the communication of the diagnosis, if circumstances allow, at around 8 years of age of the child, when he is able to understand genetic aspects and the related possible hormonal treatments.

Recommendations for parents:

1. Make the communication of the diagnosis as a process, which starts from early childhood, following the child's understanding and development.
2. Share with the child the history of his path of knowledge and acceptance of the diagnosis.
3. Maintain a clear, sincere, emotional, but not denying or minimising language of the present and future clinical situations.
4. Refer to aspects of diagnosis that children can recognise as pertinent to their life experience.

5. Encourage children to dialogue and to formulate questions for both parents and specialists.
6. Introduce themes concerning genetic aspects and infertility in the times that are appropriate to the maturation of the child (around 10 years of age), in any case before the beginning of endocrinological visits and not in adolescent age.
7. Reflect with the child on sharing this information with its life context, leaving it free to choose, but guiding it in the reflection and the ways of sharing.

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The Diagnosis of Klinefelter Syndrome at Prepubertal Age

8

Mario Mancini

8.1 Introduction

Only 6% of men with Klinefelter syndrome (KS) are diagnosed in childhood and prepubertal age. They are usually identified due to developmental delays in the genital area or otherwise due to learning and behavioral problems. The low rate of diagnosis is attributed to hard data to interpret about hormonal, testicular and penile modifications in prepubertal age, usually constantly variable and not covered by the pediatric routine investigation. Here, we will try to increase the ability of the clinician to have a precocious diagnosis of KS during this difficult age.

8.2 Clinical Markers of KS

An increased stature is common in KS boys. Some of them are above the 75th height percentile prior to 12 months from birth. More than 70% of KS patients diagnosed in childhood after 7 years of age are in a higher percentage of height and at the end of their growth are significantly taller than the predicted final target height [1]. During childhood there is a notable increase in height velocity between 5 and 8 years of age due to greater leg growth [2]. Rarely KS boys remain below the 25th height percentile [3]. There is a decreasing upper-to-lower segment ratio with age. The mean difference in height minus arm span was 2.2 ± 4.0 cm, suggesting relatively long legs in most boys [4]. A supplementary suggestion is the ratio of second to fourth finger length as a correlate of prenatal testosterone. Higher ratio seemed associated with low prenatal and reduced sensitivity to testosterone. This ratio in KS

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individuals was found higher than that in their fathers and male controls [5]. In 74% KS boys there is a fifth finger clinodactyly, in 68% hypertelorism, in 37% a high arched palate and in 35% elbow dysplasia [4]. Hypotonia is frequently observed in infants KS. Decreased muscle mass and strength have been noted in infants [6] as well as in prepubertal age [7] with Zeger et al. reporting 76% of KS boys with varying degrees of poor muscle tone [8]. In KS children followed up for 12 years, there is an undescended testis in 15% and consecutive ascending testis in 21%, resulting in a surgery procedure in 36% of the entire population. In non-Klinefelter boys in the first 3 years of life, both undescended and retractile testis reached about 8% [9].

Interesting new fields come from testicular ultrasound (US) in adult KS, finding that testes are smaller, more nodular, more vascularized, and with a major percentage of microlithiasis than those of non-KS infertile men. This US picture should lead physicians to request a karyotyping. Other works are needed in Children [10].

At peripubertal period, hypogonadism may be suspected by a late appearance of body hair and feminized distributions of pubic hair. A poor muscle mass followed by an increased fatigue and abdominal fat occurs. About 20% of developmental boys manifests a gynecomastia [11]. Learning and behavior problems will be discussed in other chapters.

8.3 Hormones

In healthy neonates, the pituitary–gonadal axis is generally activated, producing pubertal levels of serum FSH, luteinizing hormone (LH) and testosterone between 2 and 6 months of the life; this is the mini-puberty [12]. In KS patients, several studies suggested a reduced testosterone during mini-puberty [13]. Hypogonadism in KS may start early in infancy [14]. Testosterone of infants with KS in the first 2 years of life was significantly lower when compared with infants without KS, indicating an impairment of the Leydig cells function [6, 15]. Although males with KS on average seem to have lower serum testosterone, there is an overlap in testosterone levels in boys with and without KS [16]. However, Cabrol et al. found that the testosterone level was above the median of controls (210 ng/dL) in only 13% of KS infants aged below 5 months. In fact, the median testosterone value in KS was 118 ng/dL [17]. Previously Ratcliffe had published 4 KS infants all below a testosterone value of 250 ng/dL without a control group, with the maximum peak reached in the second month of life [18]. Afterwards, in childhood, the hypothalamic–pituitary–gonadal axis is quiescent [19]. In that period, there are low levels of testosterone, although they were abnormally low for the observed LH values [20]. Zeger described in KS children between 2 and 14 years a 75% of patients with testosterone levels <25th percentile for age and Tanner stage [8]. At peri-puberty of KS, testosterone is sufficient for the development of primary sexual characters, but this development usually occurs 3–4 years later when compared to normal boys [21]. Furthermore, during mid-puberty of KS boys, there is a gradual increase in luteinizing hormone (LH) and follicle stimulating hormone (FSH). Gonadotropins were found normal [6, 13] or elevated [22] in the first 2 years of life. The same results occurred between 2 and 14 years, showing normal [7] or elevated FSH and LH in 30–40% of KS patients [8]. In young adults, a rise in FSH shows the hyalinization of the seminiferous tubules.

8.4 hCG Test

Human chorionic gonadotropin (hCG) stimulates Leydig cells within testicular tissue, where plasma testosterone production increases [23]. In healthy men, a single injection of 1500 IU hCG increased testosterone levels for 4–5 days with a maximum value reached after 3 days [24]. Some evidences showed that blood testosterone levels in adult KS patients are significantly lower than those in eugonadal men. hCG administration of 1500 UI for 3 days resulted in a significantly lower increase [25]. To better quantify the difference, Belli matched KS and control adult patients showing in healthy men a rising of testosterone after a single dose of 5000 IU hCG about 90% by collecting blood samples after 5 days. In KS adult group examined in the same conditions, the raising of testosterone after 5 days from hCG was about 25% (not significant). Therefore, total testosterone increased significantly after stimulus in both KS and control patients, but its level fell from the third day in KS patients and remained elevated in the control group [26].

These data seem to show the testosterone level after the hCG injection as the best indicator of the reduced secreting potential of Leydig cells in adult KS [27]. This response does not change using different laboratory methodologies [28]. An interesting work published by Zivkovic in cryptorchid children non-KS aged 1–7 years found a strong relationship between the testosterone response after two injections of hCG 1500 UI and the germ-cell maturations within the testis. The testosterone level in boys with a good germ cell maturation ranged from 101.9 to 297.1 ng/dL and those with an insufficient cell maturation ranged from 14.6 to 184.6 ng/dL [29]. Being germ cells in KS progressively compromised, and hCG testing could be promising in KS children to estimate the degree of damage in the tubular germ cell line. Interestingly, after hCG in adults with KS, the plasma 17-OH progesterone increased more than testosterone, leading to a statistically significant rising of the 17-OH progesterone to testosterone ratio expressed in ng/100 mL (range, 0.29–0.67), whereas this ratio remained unchanged in the control subjects (range, 0.14–0.26). Therefore, a short-term hCG stimulation may reveal also an enzyme impairment of the biosynthesis of testosterone in Klinefelter syndrome [30].

8.5 Penis Length

In KS adults, 25% of patients exhibit a reduced penile development although it is rare in healthy adults [31].

Micropenis refers to a penile length smaller than 2.5 standard deviations (SD) below the normal values [32].

Its incidence was reported as 1.5 in 10,000 (0.015%) male children [33]. The length was measured when the penis is fully stretched with the foreskin retracted. The measurement has to be taken from the pubic bone to the distal tip of the glans over the dorsal side. The pubic fat should be pressed. In the first months of life, there is a rapid increase in penile length in parallel to the testosterone rising of minipuberty [34]. In the first day after birth, the penile stretched length corresponding to

the −2.5 SD value was 2.9 cm in Akin and 2.19 cm in Kutlu. Kutlu believes that micropenis should be defined below the third percentile or − 2 SD (3 and 3.02 cm, respectively), similar to the definition of short stature [35, 36]. Later, penile length increased from birth (3.49 ± 0.4 cm) to 3 years of age (4.53 ± 0.51 cm). The lower limits of the normal range (−2.5 SD) for age were 2.49, 2.67, 3.05, and 3.26 cm for 0, 3, 18, and 36 months, respectively [34]. Mean penile lengths and percentiles were shown in preadolescents also, where the length −1 SD in flaccid state was found at least 5.5 cm before 10 years of age [37]. Initially, two of the five KS infants (40%) diagnosed postnatally by Lahlou had micropenis [15]. Lee confirmed a 28% of micropenis prevalence in KS infants out of a series of seven infants [38]. Otherwise, Ratcliffe found that at birth a minority of the KS infants had a genital underdevelopment. By the end of puberty, the penis was reduced in 23% of patients [2]. Recently, Akcan et al. described an isolated micropenis in 8.7% of 23 KS children [39]. A good clinical comparison in prepubertal population is possible about Zegher where KS penile length was 4 ± 0.7 cm (3.3–4.7 cm) between 2 and 10 years versus normal children at the same age reported by Cinaz (4.9–7.45 cm) and Custer (4.2–7.3 cm) [8, 40, 41].

Penile length was found reduced also in the series of 35 KS boys and men reported by Zinn, which included 12 infants. Interestingly the authors found a negative correlation between penile length and the length of androgen receptor gene (CAG) [42]. In healthy men also, Lim et al. found a greater CAG length if severe genital defects were present, compared with controls [43].

8.6 Testicular Volume

In early infancy, testicular tissue consists mainly of Sertoli cells. They constitute the main factor together with length and diameter of seminiferous tubules determining the volume. Testis measurements could be done by orchidometer or ruler or by ultrasound (US). The orchidometer and the ruler are known to overestimate testicular volume as they measure not only the testis but also the epididymis and the skin. The best measure of the testis is performed by ultrasound. Many studies have been realized by using the Prader orchidometer.

By orchidometer, Goede found that testicular measurements, in the first 2 years of life, were between 1.16 and 2.07 mL. Later between 3 and 9.9 years the testis volume was between 1.05 and 3.33 mL and in peripubertal age, between 10 and 14.9 years, it was between 1.69 and 22.02 mL. KS patients, in the first 2 years of life, have been found to present borderline small testes. The Prader volume showed a variation of 0.5–1.5 mL [6]. Later, measurements by orchidometer between 3 and 9.9 years were 1.2 ± 0.4 mL (0.8–1.6 mL) and between 10 and 14.9 years were 2.6 ± 1.3 mL (1.3–3.9 mL) [8]. In a longitudinal study where KS patients have been followed up until the onset of puberty, at mean 11.9 years, the testes enlarged to around 5 mL volume, except for two cases in which there was enlargement to 12 mL, followed by involution [2].

Others described a possible testis enlargement of no more than 10 mL and then a decreasing to <4 mL [7].

The reduction in testicular size, noted prior to puberty, suggests an early reduction of seminiferous tubules structure and fertility. Slow degeneration starts during childhood and increases during puberty when it ends up in a widespread hyalinization and fibrosis, characteristic of KS [22]. We can synthesize that in KS there is a clear reduced testis volume measured by orchidometer. Unfortunately, there is an overlapping of these measurements with those found in healthy children or peripubertal boys. Furthermore, Prader is clearly less accurate than US. Therefore, at the moment, due to the absence of US data in KS population, it is reasonable to suspect an abnormal testis growth by using as cut-off the lower US values in normal patients (mean ± SD) taken from Kuijper or Goede. Kuijper found a mean testicular US volume increasing in the first 5 months from 0.25–0.29 mL to 0.41–0.47 mL; thereafter, the volume decreased to 0.29–0.33 mL at 9 months of age [44]. Goede reported testicular measurements with a US variation in the first 2 years of life between 0.35 and 0.55 mL. Between 3 and 9.9 years the US dimensions were between 0.36 and 1.25 mL. Between 10 and 14.9 years they resulted between 0.46 and 11.42 mL [45].

8.7 Histology

At fetal age, the number of germ cells in KS was found similar [46–48] or reduced [49, 50] respect to control samples. Signs of fibrosis were not present. In early childhood and in boys with KS, spermatogonia were reduced and the Sertoli and Leydig cells appeared normal [51]. At that point a slowly degeneration of germ cells starts, leading to a significantly reduced number of germ cells before puberty [52–54].

During the puberty, this process continues following the activation of reproductive hormones. They should have to switch from mitosis to meiosis process for gamete production. Unfortunately the majority of germ cells in KS disappear at the mitotic stage of spermatogonia or at the level of spermatocyte, during earlier differentiation and the tubules end for containing Sertoli cells only. The key moment to understand the testicular damage seems to be the early puberty when the scientific data are still controversial. Wikstrom et al., in a study of 14 KS boys aged 10–14 years and biopsied before and during puberty, found a markedly reduced number of spermatogonia and no post-meiotic spermatids appeared [55]. In 19 KS between 12 and 20 years, a mean of 1.8 spermatogonia/mm^3 was found. Very low respect control patients were aged 6–14 years containing a mean of 100.3 spermatogonia/mm^3 [56]. From the age of about 14 years, a relative Leydig-cell insufficiency occurred. As serum-testosterone levels increased, occurred an increased LH level, followed by depletion of germ cells, hyalinization of the tubules, degeneration of the Sertoli cells and hyperplasia of the Leydig cells [55].

8.8 Predictive Markers of Sperm Retrieval in Prepuberal and Peripubertal Age

Generally, in adults with KS, factors such as hormones (serum FSH, LH, free and total testosterone, E2, inhibin B, SHBG, prolactin), testicular volume and testicular histology seem to be deprived of any predictive value for sperm retrieval. Other studies at the moment highlighted that, in males with Klinefelter syndrome, the only predictive factor for a successful sperm recovery seems to be the testicular histopathology [57]. Recently, a large study in adult infertile azoospermic patients, including KS, has identified higher serum testosterone levels and lower levels of LH and FSH as positive predictive markers, but the predictive value of serum testosterone levels in KS remains unclear [58]. Rohayem et al. described a combination of total serum testosterone above 216 ng/dL and LH levels below 17.5 U/L, to result in higher retrieval rates of spermatozoa by micro-TESE in both children and adults with KS [59]. Madgar et al. reported the predictive value of high response hCG test in adult KS patients with successful sperm retrieval by TESE. In this group, the mean serum testosterone level was 1600 ± 630 ng/dL different from the failure group with 670 ± 560 ng/dL. A possible predictive testosterone value could be above 1000 ng/dL after hCG test. Testicular volume and basal testosterone were predictive too for successful picking up of the spermatozoa [60]. SHBG was not different from normal karyotype patients. The level of inhibin B is known to increase before puberty, when it is known to be regulated by Sertoli cells. After the onset of puberty, the inhibin B production becomes germ-cell dependent and can be their marker also. Therefore, as the testosterone level increased in KS boys, germ cells degeneration can be followed by a progressive suppression of the inhibin B level, becoming undetectable at the end of puberty. It could be used as marker of germ cell impairment [12]. Salehi found in adult azoospermic patients, including KS, that not only higher levels of serum testosterone and lower levels of FSH and LH, but also higher testicular volume were predictive for successful sperm retrieval [61]. On the other hand, Wilkstrom et al. reported that depletion of germ cells was associated with an increase in testicular volume. Biopsy of peripubertal boys (10–14 years old) with testicular volume < 2.0 mL showed spermatogonia of adult type, whereas older boys with testes >2.0 mL exhibited no germ cells [55]. It is likely that testicular volume might be useful as a marker of sperm recovery only when combined with hormonal pattern and age of KS boys. However, it is not clear if it is better waiting or not the increasing of testicular volume during pubertal development. A precocious testicular damage could be found by an accurate testicular ultrasound. Rocher described, in adults KS older than 21 years, a different distributions of ultrasound signal, with coarse or nodular patterns in the KS men, and slightly striated patterns in the healthy patients [10]. Other studies are needed to explore the ultrasound role in KS children and prepubertal boys.

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The Pediatric Management of Klinefelter Syndrome: What to Do and When from Infancy to Puberty

9

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Birth defects have a prevalence of 3% among live births and 25% of these "children with special needs" develop physical and/or mental disabilities, which require a very demanding medical approach. About 0.5% of these patients have genetic syndromes that may be severe, like Cornelia de Lange syndrome, or mild like Klinefelter syndrome (KS) but all can prevent personal autonomy, with important effects on assistance and social life. Hence, Italy has developed the branch of "*Pediatrics of disability*" which doesn't *only* involve the multi-specialistic medical approach, but is enhanced *also* by multi-sectorial approach which includes educational, scholastic and social elements. The pivotal professional of the welfare network of SK child is the family pediatrician who evaluates his initial abilities (functional diagnosis) and becomes familiar with the syndrome, its course and possible complications, such as mediastinic tumors in children or emergencies, such as thrombosis in later ages. He or she is also to guarantee the correct nutritional intake of the child and the normal growth and sleep, and to promote physical activity and vaccinations. Finally the pediatrician must assess if the family receives a proper social support, discovers if the child is harmoniously integrated in the class, if he is carrying out rehabilitation and if there is interaction between the facilities that assist him, to facilitate the child's participation to social life as well as possible [1].

KS has an estimated prevalence of about 1/660 males, but is considered undiagnosed in 65% of cases, while it is detected in prenatal diagnosis in 10%, in childhood-adolescence in 6% and in adult age in 19% of cases [2]. Children may be diagnosed for underdeveloped genitalia, hypospadias, undescended testes and learning and/or behavioral problems, adolescents for microrchidism, gynecomastia or delayed puberty, and adults for hypogonadism or infertility.

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The increase in gene expression of the X chromosome (*SHOX gene* etc.), or the length of CAG repeats in AR gene, and a different methylation pattern and a non-random inactivation of X are the pathogenetic factors of the multiple clinical symptoms of KS. They affect mainly the testicular function determining a gonadal dysgenesis and a hypergonadotropic hypogonadism, with pervasive effects on other hormonal levels and on metabolic, cardiovascular and sexual functions, including fertility, generating a vicious cycle that increases insulin resistance and fat accumulation, influencing the body composition and the risk of osteoporosis [3, 4].

9.1 Clinical Picture and Diagnosis at Different Ages

The clinical picture of KS is extremely variable, indeed the characteristic phenotype of the hypogonadal eunuchoid boy is less frequent of his normal appearance. This variability depends on the association of chromosomal aneuploidy with a polymorphism of the AR-gene (Androgen Receptor, located in Xq11-q12) represented by an exaggerated repetition of a CAG triplet in the exon 1, that makes the receptor insensitive to androgens and correlates with the severity of the phenotype. Basically, testosterone-related symptoms don't occur during childhood, whose typical signs such as speech disorders or longer legs, depend on genetic anomaly rather than hypogonadism. Hormonal deficit is silent until pubertal onset, in which testosterone is generally sufficient for the development of body and genitalia, in fact KS adolescent testes grow to a normal initial volume of 6 mL, but it shows up later, after the age of 25 in 75% of KS patients. Consequently, the adolescence is the best period to pursue testicular sperm extraction (TESE) procedures for sperm cryopreservation, prior to the spermatogonial apoptosis due to testes degeneration.

The adult constant clinical sign is microrchidia (<10 mL in adulthood), while azoospermia can be detected in 92% of patients in case of infertility. Therefore, the small clinical relevance of these signs induces subjects, who show no other defects, not to seek medical attention.

In pediatric age the clinical signs, useful for diagnosis, are even more rare and non-specific (Table 9.1).

The KS *newborn* has no distinct dysmorphic features, with familiar facies and normal external genitalia, which do not allow diagnosis at this age. The syndrome can be suspected if cryptorchidism, especially if associated with hypospadias and/or micropenis (length <2.5 cm at birth), is present.

Table 9.1 Diagnosis of KS by age

Age	Clinical features
0–3	Hypospadias, micropenis, cryptorchidism
4–6	Language delay
7–11	Language delay, learning difficulties, behavioral problems, reduced testicular volume
11–18	Delayed or incomplete puberty, eunuchoid habitus, gynecomastia, reduced testicular volume
Adult	Infertility, reduced testicular volume, hypogonadism

In *subsequent years*, somatic growth is normal, although generally after 8 years, an increase in the length of the lower limbs is observed, probably due to the overexpression of the *SHOX gene*, located in the PAR1 region of the supernumerary X chromosome, which escapes inactivation and is involved in maturation and skeletal growth.

The problem of psychomotor development in KS has been much debated since the first reports based on selected studies, carried out on a reduced scale, showed the association with mental retardation which, instead, was subsequently found with the same incidence of the general population (3%). More precisely, over time it has been observed that there is no general reduction in intellectual capacity, but there may be a sectoral flaw in some specific functions concerning, in particular, language and executive functions, as observed in 40% of patients [5].

Samango Sprouse described the "cognitive profile" of these children, highlighting first of all the presence of a normal IQ (70–110) and a delay in the acquisition of the stages of the language, defining them as "late talkers" (first words at 20 months–4 years). KS children reveal a characteristic cognitive and linguistic profile, with a notable dissociation between language skills, which are deficient, and visuo-spatial skills which are instead preserved. A gap between linguistic IQ and performance IQ is therefore possible, with an obvious reduction in the overall IQ, which is calculated in 15 points for each supernumerary X chromosome. *Developmental dyspraxia* is a disorder of the development of motor function both in the programming and in the execution of an intentional movement, aimed at achieving a purpose, while there is no compromise in the execution of automatic movements. In practice, KS are clumsy children, with difficulties in organizing work and in carrying out instructions, but through continuous practice they can acquire functions and carry out daily activities without great difficulty. They often show difficulties in both coarse and fine movements, in fact verbal dyspraxia is often associated with oral–facial dyspraxia, that is, a difficulty not only in programming joint movements, but also in voluntary fine non-verbal movements with the oral–glosso–pharyngeal musculature (e.g., blowing, pulling a kiss, swell cheeks). However, the spontaneous motricity of these muscle groups is preserved. In practice, verbal dyspraxia involves a poorly intelligible and not fluent expressive language and these difficulties tend to persist over time. Disorders of language production and processing, if neglected in preschool, can lead to difficulties in reading and writing. Hence it's clear the importance of early identification of these difficulties, with the aim of planning an early rehabilitation.

At about *3 years of age*, the KS child must undergo a consultation by the child neuropsychiatrist in order to diagnose any motor dyspraxia and send the child early to rehabilitation of language through speech therapy.

In *school age* these children may have specific learning disorders (school difficulties, reading difficulties and dyslexia) as well as behavioral disorders, motivated by low self-esteem, linked to language problems. Early intervention allows these children to solve the problem of language in preschool, so that they can face entry to school with all the prerequisites for adequate school learning and for a peaceful socialization with peers [6, 7].

The tests used for speech development are: F.L.T. FIRST LANGUAGE TEST (Understanding) *from 1 to 3 years* and L.E.T. Language Evaluation Test (Understanding, Production, Morphosyntax) as second administration *from 2.5 to 6 years*. The tests used for dyspraxia are APCM-2—Praxic and Motor Coordination Skills *from 2 to 8 years.* TNA—Neuropsychological Test of Apraxias for the developmental age *from 3 to 11.6 years.* The WISC-IV scale (Wechsler Intelligence Scale for Children) is the best clinical tool to assess cognitive skills individually with children aged *between 6 years and 0 months and 16 years and 11 months*.

At *puberty,* testes begin to enlarge but, in KS boys, reach a peak volume of no more than 10 mL and then decrease down to smaller than 4 mL, so this low testicular volume, the pubertal delay, the modest development of secondary sexual characteristics and the appearance of gynecomastia that can give, especially to overweight patients, a gynoid appearance also for the limb root fat deposition, must be carefully evaluated. Furthermore, at puberty, the bi-iliac diameter, the waist and hip circumference, the abdominal and total fat mass and the body mass index (BMI) are increased, related to the reduced testosterone levels, since childhood. If the abnormalities of sexual and/or psychomotor development are detected early, it's possible to set up a clinical follow-up to prevent or intercept promptly the disorders related to the condition. In fact, it is the pediatrician's job to know the health issues of the adult SK patients and identify their warning signs in the pediatric age, distinguishing specialistic problems from the pediatric ones (Table 9.2).

Table 9.2 Clinical management of KS child

Genetic evaluation	– If prenatal diagnosis, postnatal confirmatory karyotype for mosaicism – At the time of diagnosis: genetic counselling
Auxological evaluation	Every year: – Weight and height (percentiles) – Growth rate and body mass index – Nutritional status and waist circumference (>9 years) – Blood pressure (>9 years) – testicular volume (mL) – Palpation of breast tissue for gynecomastia (>10 years) – Vaccinations status
Endocrinological evaluation	– At 3 months (mini-puberty) FSH, LH and testosterone serum levels – From 6 years old if micro-penis, otherwise >9 years – At the start of puberty: Serum gonadotropins and testosterone every 6 months – Semen analysis (14 years)
Neurodevelopmental evaluation	– From 3 years, then every year if problems WISC-IV + APCM-2 scale – Speech evaluation in preschool age: FL Test/ LETest → speech therapy
Cardiologic evaluation	– Newborn and >10 years: ECG and echocardiogram
Pneumological evaluation	– Sleep study if sleep apneas
Dental evaluation	– At age 5 and then at age 13

Table 9.2 (continued)

Orthopaedic and bone metabolism evaluation	– At 8 years and then if necessary – Dietary intake of calcium and vitamin D supplementation (400 UI/day) and random check of vitamin D
Neurologic evaluation	– If speech delay or postural tremors or atypical movements – If seizures: means elecroencephalogram (EEG)
Autoimmunity evaluation	– At age 10: Thyroid function screening every 2 years, with thyroid ultrasound (US) if abnormal – Monitoring of symptoms of autoimmune disease
Psychologic evaluation	– Parents at prenatal diagnosis and then child when necessary (communication of diagnosis)
Dyslipidemia and NAFLD and insulin resistance	– From 9 years and then every year (more if family risk or obesity). Cholesterol screening with lipid panel, transaminases, HbA1C – From 9 years and then every year hepatic US
Evaluation of malignancies risk	– Chest X-ray or computed tomography (CT) to rule out mediastinal mass if symptoms of cough, dyspnea, or chest pain – Serum beta-HCG and alpha-fetoprotein if it is necessary

9.2 Pediatric Problems in KS Children and Their Monitoring

The most common feature of KS is the insufficiency of testicular development that seems to be abnormal from very early in life. In fact, normally, the hypothalamic–pituitary–testicular axis is activated during the second trimester of fetal life, the first 3 months of life (mini-puberty) and the puberty, while it is quiescent during childhood. Hypogonadism in KS may start as early as fetal life or early infancy, so, if micropenis is present *in the first 6 months*, an endocrinological evaluation is necessary, with a urologic consultation if cryptorchidism, inguinal hernia and urogenital malformations (hypospadia) are present, too.

Subsequently an endocrinological visit is indicated *at 10 years* (male puberty starts), with a serum dosage of follicle-stimulating hormon (FSH), luteinizing hormon (LH) and testosterone, to be repeated every 6 months and physical examination should include palpation of breast tissue to detect gynecomastia. The endocrinologist should decide also when or if to start androgen replacement (see Chap. 13).

In addition to hormonal problems, KS also has some pediatric comorbidities, resulting in life expectancy being reduced by 2–6 years, compared with 46,XY men (Table 9.3).

Several studies show that adult SK patients have a greater tendency to develop insulin resistance (38%) and related disorders such as early type 2 diabetes mellitus, non-alcoholic fatty liver disease (NAFLD), increase in serum total cholesterol, LDL and triglycerides and HDL reduction, altered body composition with increased fat mass and reduced lean mass and *metabolic syndrome*, that is a constellation of signs, including large waist circumference, dyslipidemia, elevated fasting blood glucose and high blood pressure (50% of adult patients). Further studies have associated metabolic alterations with the reduced testosterone levels, typical of KS adults, observing an inverse correlation between serum hormone values and insulin

Table 9.3 Features and comorbidities of KS

	Features	Frequency (%)
Children	Learning disabilities	>75
	Abdominal adiposity	~50
	Metabolic syndrome	46
	Delay of speech development	40
	Increased height	30
	Cryptorchidism	27–37
	Psychiatric disturbances	25
	Congenital malformation, cleft palate, inguinal hernia	~18
	Type 2 diabetes	10–39
	Decreased penile size	10–25
	Osteopenia	5–40
	Mediastinal cancers	Increased risk (~500-fold)
Adults	Small testes	>95
	Increased gonadotropin levels	>95
	Azoospermia	>95
	Infertility	91–99
	Decreased facial hair	60–80
	Decreased testosterone levels	63–85
	Decreased pubic hair	30–60
	Gynecomastia	38–75
	Osteoporosis	10
	Mitral valve prolapse	0–55
	Breast cancer	Increased risk (~50-fold)
	Fractures	Increased risk (2 to 40-fold)

resistance. In addition, it seems that the altered lean/fat mass ratio is also related to it. As confirmation of these hypotheses, a marked improvement in the patient's clinical picture is evident after replacement therapy, particularly when associated with metformin and/or diet [8].

A well-known study shows that androgen deficiency is already present before puberty in KS boys, although the hypothalamic–pituitary gonadal axis is quiescent during childhood and, therefore, the evaluation of LH, FSH and testosterone concentrations generally has a low clinical value [9]. A recent work has highlighted in the adult patient a strong correlation between low testosterone levels and the development of NAFLD [10].

These observations on KS adults, induced to focus the attention on the metabolism of KS children, demonstrating an increase in LDL in 37%, insulin resistance in 24% and metabolic syndrome in 7% of pediatric cases, all related to hypogonadism [8]. In fact, low testosterone levels can be predictive of abdominal fat increase, and together promote metabolic syndrome and type 2 diabetes, which are in turn associated with an increase in cardiovascular risk, related to a marked tendency to obesity also in pre-pubertal age. These are the consequences of the imbalance between testosterone (low) and estrogens relative increase (T-E ratio), that favors the storage of abdominal fat, despite which a normal BMI is maintained due to the reduction in lean body mass. For these increased risk factors in KS children, it's mandatory to follow recommendations of the American Academy of Pediatrics to *screen*

cholesterol and fasting lipid panel at the age of 9, 11 and after puberty. Moreover, to prevent metabolic alterations, it is essential to control the dietary intake of simple sugars (fructose) and proteins (0.7–1.1 g/kg/die) and to promote daily physical activity.

Finally, during immobilization for surgery or venous catheterization, it's useful to consider prophylaxis of thromboembolic and peripheral vascular disease.

Bone metabolism and structure in KS adults are influenced by testosterone that stimulates bone mineralization both indirectly, through aromatization in estrogen, and directly, binding to the androgen receptor of osteoblasts, therefore hypogonadism reduces bone density in combination with the extra-gene dosage of supernumerary X chromosome. Rare studies on KS children and adolescents report normal bone density, consequently, in prepubertal age, it's useful to *ensure adequate calcium intake and vitamin D supplementation* (400 UI7die), regular physical exercise and normal BMI, without evaluating bone mineral density and in post-pubertal age to maintain normal sex steroid levels [11].

In adults with KS a higher incidence of *autoimmune diseases* has been described, also correlated with the imbalance of T-E ratio, in fact, in post-pubertal age, Hashimoto's thyroiditis may develop, with consequent hypothyroidism, as well as chronic juvenile arthritis, systemic lupus erythematosus, Sjogren syndrome, multiple sclerosis, Addison disease and type 1 diabetes mellitus. No studies evaluated autoimmune diseases in KS children, so it's recommended to evaluate only if suggestive symptoms appear [12].

Although the overall incidence of malignancy is not higher in the KS adult population, there is an increase in multiple types of cancer (*neoplastic* risk of 1–2%).

Regarding breast cancer, 4% of patients have KS, with a 20 times higher risk and earlier onset (58 years). It can depend on the imbalance of T-E ratio, favored by an increase in the aromatization of androgens in the adipose tissue of obese patients [13].

The risk of extragonadal germ cell tumor is increased 500 times in KS patients (0.1% of KS patients) who are 8% of subjects with this primary cancer. Half of this tumors occurs in adolescence more than in childhood and manifests with precocious puberty, cough, dyspnea or chest pain (mediastinic location), and needs a chest radiograph or computed tomographic (CT) scan, with beta-human chorionic gonadotropin (HCG) and alpha-fetoprotein serum dosage.

In addition, KS boys showed an association with non-Hodgkin lymphoma that needs confirmation in further studies. No routine neoplastic screenings are recommended in childhood, but suspicious symptoms should be carefully evaluated [14, 15].

Other less frequent medical conditions that occur in KS children more than those in general population are congenital anomalies like inguinal hernia, congenital heart and kidney malformations, cleft palate with velopharyngeal insufficiency and dental anomalies like taurodontism and susceptibility to caries. Different studies reported a predisposition to seizures in KS boys with a prevalence of 15%. Tremors are also considered frequent in this condition [16–19].

In conclusion, it is important to carry out also some *psychological* consultations in various phases of KS life, starting from parents support after prenatal diagnosis,

to facilitate the acceptance of the unborn child and then directly to the child or boy, to explain the reasons for repeated medical checks, the meaning of KS in general and the specific problems of fertility with their solutions [20–23].

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Klinefelter Syndrome: From a Disabling Condition to a Variant of Normalcy: Neuropsychiatric Aspects

10

Michele Fabrazzo

Klinefelter syndrome (KS) is characterized by a broad spectrum of clinical manifestations that include several phenotypes such as hypogonadism, androgen deficiency, and infertility [1]. Further phenotypes are distinguished for being associated with behavioral or psychiatric disorders. Also, neuroanatomical, cognitive, and behavioral abnormalities have been examined in the clinical research, primarily focused on patient's physical phenotypes [1, 2]. In some KS patients, the extra X chromosome is associated with cognitive, psychosocial, motor, and language deficits. KS patients show cognitive deficits even before puberty when testosterone levels are nearly normal, so the profile is unlikely to result from androgenic insufficiency affecting neural development. However, a number of anecdotal studies affirm that testosterone supplementation leads to preserve more effectively gray matter in the superior temporal gyrus [3].

The neuropsychological phenotype in adults affected by KS is highly variable. Impaired measures of verbal skills, high incidence of dyslexia, and social dysfunctions are among the most consistently reported behavioral phenotypes [4]. The verbal area compromised encompasses delay in early language development, learning disabilities in reading and spelling, difficulties in the production of syntax, word retrieval, and nonsemantic cues in spoken language [5]. As a consequence of such impairments, young KS patients frequently necessitate speech and language therapy, as well as specialized help at school [6]. Disabilities in verbal cognitive function may explain the reason why KS adult patients predominantly have a lower educational level. The general cognitive abilities appear to be close to the norm, and yield a mean intelligence quotient (IQ) of 87.9–110 [4]. Further cognitive problems include impairments in verbal and nonverbal memory and in executive functions [7].

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Visual and spatial cognitive abilities appear normal in adults with KS, and arithmetic abilities normal or mildly impaired [8].

The most prominent behavioral problems in KS patients occur in the social domain, which comprises social withdrawal, social anxiety, shyness, impulsivity, and inappropriate or antisocial behavior [4]. In early adulthood, KS patients report having few or no friends, scarce energy and initiative, few or no spare time interests, and poor relations with siblings and parents [9].

Reduced social functioning has been attributed to language-based learning difficulties, social cognitive impairments, and verbal disabilities [5]. KS patients may show significant differences in specific domains of social behavior, whose frequency does not appear different compared to the general population. In this type of patient, characteristic features are reported, such as diminished engagement in social behavior implicating negative emotions, which manifest in refusing a request or standing up for one's rights in public.

In addition, XXY patients present high rates of autistic-like traits across all the dimensions of the autism phenotype, specifically in the five domains of social skill, attention switching, attention to detail, communication, and imagination. Difficulties in coping with social situations, principally high levels of distress during social interactions, are consistent with reports of social anxiety, social withdrawal, and shyness in individuals with the XXY karyotype [10]. Children or adolescents with KS may primarily manifest difficulties in social adjustment, which can perdure until adulthood, and social distress, more prevalent compared to the reduction in general engagement in social behavior.

The reported verbal disabilities include impairments in both language production and perception and indicate compromised language functions that are typically located in the left hemisphere [4]. Young and adult KS patients show a range of disabilities in reading, articulation, phonemic processing, spelling, language expression, verbal memory, language and word comprehension, word retrieval, and verbal expression of thoughts. Such impairments determine a verbal IQ slightly lower than performance IQ [4]. Specific language dysfunctions have also been associated with morphological abnormalities of the temporal lobe [4]. Furthermore, structural magnetic resonance imaging (MRI) studies in KS patients have indicated a reduced volume of the regions belonging to a neural network involved in social cognition, such as the amygdala, the insula, the anterior cingulate cortex, and the superior temporal gyrus [2, 3]. The difficulties in social adaptation, together with the structural brain abnormalities associated with the XXY karyotype, suggest that a genetic mechanism involving genes on the X chromosome might lead to disturbances of social cognition in KS patients.

Social coping problems may indicate an increased vulnerability to developing traits and symptoms that pertain to the autism or schizophrenia spectrum.

The outcomes of current studies suggest a more elevated risk of psychiatric disorders among individuals with XXY, as assessed by most psychiatric interviews [11]. Despite the limited samples analyzed in a part of studies, psychiatric disorders resulted were prevalent in a higher number of patients compared to the general population. Individuals affected by KS indeed have a higher risk of developing

psychiatric disorders, although the emerged symptoms are described as heterogeneous, atypical, and frequently transnosographic [12].

A review of studies on male psychiatric inpatients from the 1960s to the 1990s indicated a 0–48% frequency range of KS in schizophrenic patients [10] and a four- to fivefold increase in KS prevalence compared to the general population. Prospective studies on KS also reported higher rates of psychiatric referral among children diagnosed with KS and 54% of KS males receiving a diagnosis of mild to moderate psychiatric disorders even during adolescence [13]. A register study reported that individuals with KS have a 3.65% increased hazard ratio (95% confidence interval, 2.92–4.55) of being hospitalized for a psychotic disorder [14]. Studies on KS boys and adults unselected for psychiatric disorders support such findings, showing an increased prevalence of schizotypal traits, schizophrenia symptoms, and disorders that comprise psychosis, depression, anxiety, autism spectrum, and attention deficit/hyperactivity (ADHD) [15]. MRI studies on KS patients reported smaller whole brain volumes, enlarged lateral ventricles, and volume reductions of the superior temporal gyrus, amygdala, hippocampus, insula, and cingulate cortex [2]. Such brain areas were equally damaged in patients affected only by schizophrenia, as emerged by the comparison between the two types of patients. However, no systematic reports of psychotic psychopathology have been analyzed in large samples of KS patients unselected for psychiatric disorders.

A few studies [3, 15] suggested that the 47, XXY karyotype is closely associated with high levels of schizophrenia-spectrum pathology. Notably, the mean level of schizotypal traits, measured by the Schizotypal Personality Questionnaire (SPQ), was significantly higher in KS patients than in healthy controls. Similarly, the Positive and Negative Syndrome Scale (PANSS) scores showed increased levels of schizophrenia symptoms in the KS group. A crucial role for X chromosome abnormalities in the etiology of schizophrenia has been presumed [16]. Abnormal cerebral lateralization may be liable to develop schizophrenia, possibly involving an abnormal expression of a gene on the X chromosome, which directs the development of cerebral asymmetry [17]. Stemkens et al. [18] found that impaired speech and motor developmental delay occurred significantly more often in KS patients with a paternal X chromosome compared to those with a maternal extra X. Interestingly, two studies investigating the 47, XXY karyotypes in a sample of patients diagnosed with schizophrenia evidenced another support for a link between X chromosome aberrations and liability to schizophrenia [15, 19]. Furthermore, KS patients have reported an abnormal cerebral asymmetry and a prevalence of the genetic disease of 0.1–0.2%, which instead might be several times higher in patients with concurrent KS and schizophrenia [1]. Another study also reported an incidence of auditory hallucinations in 4 out of 11 KS patients [20]. Overall, the risk of autism, psychosis, and ADHD showed an increase in behavioral problems and social/relational difficulties, which may have contributed to exacerbating the disease.

Anxiety and depression are two of the most prevalent and debilitating affective symptoms among KS patients [4]. The range of clinical depression rate is 19–24%, and approximately 18% of KS patients suffer from generalized anxiety. A total of

310 adolescents with KS showed a 68% rate of prevalence of depressive symptoms [13].

The literature has rarely reported associations between bipolar disorder and KS [21], a topic covered by Delavenne et al. [22]. The authors described a clinical case of a patient with a psychiatric history of one depressive episode and at least two hypomanic episodes, in addition to a family history of two relatives diagnosed with bipolar disorder, which would suggest that the patient as well was affected by type II bipolar disorder.

In a retrospective observational study, Aksglæde et al. [23] reported that eight adult patients were medically treated for depression, two for anxiety, and two for psychotic conditions. In addition, the authors described two cases of ADHD and one case of atypical autism.

KS patients often show low self-esteem and closure added to anxiety, mood disorders, and problems of socialization [4]. Zeger et al. [24] pointed out that the source of anxiety and mood disorders is originated by school problems, linked to the impossibility of achieving satisfactory results, and poor socialization, often caused by derision from peers. Considerable variability of symptoms emerges, however, in the literature [24, 25].

The related etiology, manifestations, and consequences of depression in individuals with XXY karyotype are still scarcely known. It remains unclear whether depressive symptoms are primary, secondary, or, instead, both components of KS, deriving by a disabling disease.

On the whole, research on KS patients shows that depression has a significant negative effect on the quality of life [26], being a leading cause of disability and a major risk factor for suicide [27]. A recent study [28] pointed out that the quality of life outcomes (well-being, body image and self-esteem, mental and general health) are considerably inferior in KS subjects compared to those of the general population. In patients affected by KS, physical quality of life is significantly associated with a higher employment rate and income, a more intense physical activity, and smaller daily consumption of drugs in addition to testosterone therapy. Remarkable independent predictors of a better mental quality of life, instead, are found to be higher income and living with a partner [4].

An association between hypogonadism and depression has also been suggested, although study results are yet inconsistent. One population-based study reported a hazard ratio of 4.2 for depression among hypogonadal men [29]. Similarly, another population-based study found that men affected by depression were 1.55–2.71 times more likely to have low testosterone levels than those without depression [30]. Differently, a follow-up of the Massachusetts Male Aging Study (MMAS) found that serum testosterone levels alone were not significantly associated with depressive symptoms, except among men with shorter cytosine-adenine-guanine (CAG) repeat lengths in the androgen receptor gene [31]. Results of randomized, controlled intervention trials are also controversial, with some studies of testosterone replacement in hypogonadal men showing improved mood [32], whereas at least one study found no significant difference in mood after testosterone therapy [33].

The personality and behavior of KS patients have been described as shy, reserved, sensitive, and passive in childhood, which, jointly with neglected learning difficulties, may lead to secondary adaptation and behavioral problems during adolescence [4, 34]. A cluster of more potential predictors of anxiety and/or depression in KS patients may be actually the personality traits, since they develop early in life, are stable over time, and are linked to various forms of psychopathology [35]. Increased neuroticism in the general population is usually associated with generalized anxiety disorder (GAD) and major depressive disorder (MDD), whereas conscientiousness and extraversion are negatively correlated with both GAD and MDD [36]. In other studies, personality traits have been used to identify individuals at risk for onset of mental illness [37], thus supporting the vulnerability model of psychopathology, which hypothesizes that personality traits contribute significantly to developing psychiatric disorders. Skakkebaek et al. [38] showed that KS is associated with high levels of neuroticism and low levels of extraversion, conscientiousness, and openness to experience. Additionally, the same authors [26] reported that neuroticism is the strongest (and the sole significant) mediating link between KS and the level of both anxiety and depression, besides having an impact on different phenotypes of the KS population.

More extensive research is needed to identify the clinical correlates of depression in individuals with XXY and to determine whether the manifestations or consequences of depressive symptoms pertain specifically to KS. Intervention trials are also necessary to determine whether individuals with XXY respond to antidepressants differently from individuals of the general population.

All KS patients should receive a comprehensive psychological or psychiatric assessment to manage the increased risk of anxiety, depression, and psychosis or behavioral disorders. Group psychotherapy could be beneficial to KS patients for expressing and sharing their sense of shame and isolation and for getting the support of peers. Psychological intervention as well would be helpful in executing the most profitable strategies to enhance the pharmacological compliance of KS subjects. A recent study on the quality of life of this type of patient revealed reduced well-being perceptions in patients on androgen replacement therapy [39].

The treatment of Klinefelter syndrome requires to be analyzed in more depth to ascertain which intervention, psychological and/or pharmacological, proves to be the most helpful for patients to achieve more effective coping and adaptation to XXY, as well as to ameliorate psychopathological symptoms and quality of life.

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Testicular Development

11

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Recent epidemiological studies have made it possible to better understand the phenotypic variability of Klinefelter syndrome by expanding the spectrum of causes that include genetic, epigenetic, and environmental ones. Unfortunately, knowledge of the molecular mechanisms responsible for testicular failure still remains poor. The extra-numerical X chromosome could play an important role in the pathophysiology of testicular development in the prepubertal period. Germ cell failure constitutes a hallmark, but it is not yet clear whether the defect is primarily found in the germ cells or if their maturation is compromised by an abnormal gonadal microenvironment.

More than 1000 genes that can influence gonadal development and brain growth and development map on the X chromosome. The "gene-dosage" effect of the extra genetic material poses two important questions: the difficult inactivation of the excess genetic quota and the discovery of a polymorphism of the androgen receptor gene characterized by repetitions of nucleotide triplets of different lengths [1–4].

For a more detailed view of testicular development and to understand what really happens in the different phases of life compared to what occurs in the presence of a normal karyotype, it is necessary to consider the changes that occur from birth to adulthood in the patient with Klinefelter syndrome. Normal human testicular development is a continuous process from birth to puberty. The diameter of the seminiferous tubules constantly increases up to the age of 12. Immediately after birth, Sertoli cells, gonocytes, and spermatogonia are found in the seminiferous tubule. Gonocytes occupy the central position of the tubule but tend to migrate peripherally toward the basal membrane of the tubule. Here, Sertoli cells that secrete the anti-Mullerian hormone (AMH), responsible for the regression of the Muller ducts, are also probably involved in the differentiation of Leydig cells. Once in contact with the tubule basal membrane, the gonocytes become spermatogonia.

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At the peritubular level, there is a connective tissue made of collagen and fibroblasts that are distributed in such a way as to form concentric rings around the tubules; it is precisely in this site of the testis that Leydig cells are found [5]. During puberty the development of all testicular components is completed and empty seminiferous tubules, mature Sertoli cells, Leydig cells with an evident development of the smooth endoplasmic reticulum, and germ cells (spermatogonia, primary and secondary spermatocytes, and spermatids) are found. In the early stages of puberty, gonadotropins influence changes in the peritubular connective tissue; in fact the basal membrane becomes multilayered and fibroblasts transform into myofibroblasts [5].

In men with Klinefelter syndrome, the histopathological picture detected by testicular biopsies shows a maintenance of the seminiferous tubules up to the prepubertal phase, whereas there is a numerical decrease in germ cells. However, it seems that Sertoli and Leydig cells remain normal [6]. In addition, prepubertal boys with Klinefelter syndrome have a normal pituitary–gonad function. In the pubertal phase, there is an increase in gonadotropin synthesis and a relevant decrease in inhibin B (INHB), whereas testosterone, after an initial increase, tends to fall sharply. This condition induces a process of initial hyaline fibrosis at the level of the seminiferous tubules that continues to extend into adulthood, outlining a picture of testicular atrophy. This is associated with a picture of interstitial fibrosis.

Based on literature data, the testicular volume, the histological features, and the serologic markers of gonadal function vary in different stages of life: fetal, infancy, prepubertal childhood, puberty, and adulthood.

As for fetal life, there is insufficient evidence showing a condition of hypogonadism in the fetus with Klinefelter syndrome. The studies are few and have reported the measurement of testosterone in the amniotic fluid and cord blood, but the measurements have not been carried out by liquid chromatography mass spectrophotometry, a method essential for the evaluation of very low concentrations [7, 8].

Infants with Klinefelter syndrome show slow penile growth in the first year of life and low testosterone levels compared to controls confirming a decreased androgen exposure and failure to activate the pituitary–gonadal axis during mini-puberty [9]. Testicular biopsies of infants with Klinefelter syndrome have a lower number of spermatogonia but normal histological characteristic. Luteinizing hormone (LH), and insulin-like peptide 3 (INSL3) levels are similar to those found in normal infants. The biomarkers of Sertoli function, AMH and INHB, seem to be in the normal range at this age [10–12]. Because of the variability of the timing and peak of postnatal testosterone, there is no sufficient evidence to establish whether hypogonadism occurs in infants with Klinefelter syndrome [7].

Germ cell hypoplasia is evident even though the function of Leydig and Sertoli cells appears normal in childhood. Considering that the gonadotropin secretion is quiescent in the prepubertal period, serum and follicle-stimulating hormone (FSH) levels are high and testosterone levels are in the lower part of the range in some patients with Klinefelter syndrome.

A study of 90 boys with Klinefelter syndrome showed very low concentrations of AMH (13%) and/or INHB (31%), whereas a quarter of them had very high AMH levels [7]. It is known that AMH inversely correlates with the intratesticular concentration of testosterone and its levels decrease when the androgen receptor begins to be expressed on Sertoli cells, and this is a sign of their maturation [13]. The lack of AMH decrease is a sign of testicular dysfunction. Therefore, these markers could predict the onset of gonadal failure.

Mid-phase puberty is the period when the testicular dysfunction occurs in most of the boys with Klinefelter syndrome. In detail, early pubertal boys with Klinefelter syndrome have an increase in their testicular volume by 6–8 mL and in serum LH, FSH, and total testosterone levels that reach the pubertal range [14, 15]. In the mid-stage of puberty, this hormonal situation is reversed: FSH and LH levels rise and serum testosterone levels and testicular volume decrease. Testicular biopsy shows absence of germ cells already in early puberty accompanied by other histological changes typical of Klinefelter syndrome some years later.

The onset of hypogonadism is accompanied by an insufficiency of Leydig cells, which is reflected in a drop in testosterone levels and an increase in LH levels. It has been hypothesized that testosterone appears to accelerate, in the initial phase of puberty, the reduction in germ cells and therefore a reduction in inhibin B levels by Sertoli cells with a consequent increase in FSH. Unable to initiate a normal spermatogenesis process, Sertoli cells will produce less AMH as if to signal a rapid depletion of germ cell reserves [16].

A final aspect to consider in the prepubertal phase of boys with Klinefelter syndrome is the expression of androgen receptors. In normal men, these receptors begin to appear in the nuclei of Sertoli cells in the transition phase from prepuberty to puberty. All this takes place during the hypothalamic pulse-generator phase or in conjunction with the increased secretion of FSH and testosterone [17]. In the Klinefelter syndrome, there seems to be a constant expression of these receptors for androgens, but mainly located at the cytoplasmic level [18]. Furthermore, these receptors are also expressed by the Leydig cells after the prepuberty to confirm their functional impairment and the consequent Leydig cell hyperplasia [5]. This latter aspect, associated with the hormonal and histopathological features, confirms that the anatomic–functional degeneration of the testes is accelerated from puberty onward and that the prepubertal state represents a transition toward the phenotypic expression of a chromosomal aberration with clinical aftermaths.

Beyond the aforementioned testicular histological changes, adults with Klinefelter syndrome have FSH universally elevated and LH high in most cases [14]. INHB is below the normal range and AMH is undetectable [12, 19]. Testosterone concentration can be normal or low, as well as INSL3 [20].

One of the biggest challenges is trying to understand in the context of the testicular dysfunction to what extent hypogonadism can be corrected by androgenic integration with respect to the condition of aneuploidy itself.

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The Klinefelter Puberty

12

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12.1 Epidemiology and Diagnosis

Six percent of all the diagnoses of Klinefelter syndrome (KS) are made in childhood or adolescence. The diagnoses made in adolescence are consequential to abnormal physical features such as gynecomastia and/or micro-orchidism, lack of pubertal progression, as well as poor muscular bulk and nonfamilial obesity [1].

The delay in the diagnosis is related to the time when parents first expressed concern about their child to the physician. The mean age of diagnosis is 21.1 years, that is 2 years later than when parental concern is over endocrinologic issues, which occurs at the mean age of 19.1 years [2].

12.2 Phenotype

At puberty, attention can be drawn to the possibility of this syndrome in the presence of gynecomastia or less developed testes, which may even be atrophic. The phenotype of KS at puberty is highly variable. Varying levels of androgens, testosterone (T)/estradiol (E2) ratio, androgen receptor sensitivity differences associated with variable cytosine-adenine-guanine (CAG) repeat length, or variation in X chromosome inactivation may influence phenotype and social characteristics, although other genetic mechanisms may be involved [3].

Eunuchoid proportions cannot be ascribed solely to hypoandrogenism since the increased height is documented before pubertal onset. Fusion of the epiphyses may be delayed in KS adolescents by 3–4 years, contributing to tall stature. Short stature homeobox-containing gene (SHOX), located on the sex chromosomes, is

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overexpressed due to the existence of an extra copy of the SHOX gene in KS and could be responsible for increased final height [4].

The prevalence of gynecomastia in KS during puberty is higher (from 50 to 88%) than that observed in adolescent boys (60%) and may be more likely to persist in KS [5]. The pathogenesis of the gynecomastia in KS persists to be obscure. Elevated E2 levels in KS are probably due to an increased peripheral conversion of T to E2 or can reflect increased testicular E2 secretion resulting from excessive follicle-stimulating hormone (FSH) and luteinizing hormone (LH) stimulation [6]. High E2 levels might induce an increase in sex hormone-binding globulin (SHBG), which reduces free T. These hormonal alterations may account for the persistence of gynecomastia, although KS boys show unique histologic changes such as hyperplasia of the inter-ductal tissue, not typical feature of high estrogen states [7].

12.3 Androgen Development

During puberty, in the majority of KS patients, androgen concentrations seem to be sufficient to allow the regular onset and progression of puberty until completion of epiphyseal closure and variable development of secondary sexual characteristics without any symptoms of hypogonadism.

KS adolescents start pubertal development entirely within the normal range. Delayed onset of puberty is an exceptional consequence in KS boys with more complex karyotypes. Pubertal virilization to Tanner stages IV–V is uncompromised in a high proportion of boys with KS [8].

Normal serum testosterone levels, through hypergonadotropic compensation, were reached in 62% of adolescent KS patients. After an initial physiological rise, T concentrations tend to fall to the mid-low range throughout the development process. Tanner stages IV–V in KS adolescents present mean T levels significantly lower than controls. An arrest or even a reduction in the testicular size from mid-puberty occurs almost entirely in KS adolescents. The degeneration process comes with a relative Leydig cell insufficiency as revealed by the reduction in serum T levels and increasing LH levels. The rise in serum gonadotropin is indicative of subnormal gonadal responses to central hormonal stimulation. LH levels were increased in 84% of KS patients [9].

Insulin-like peptide 3 (INSL3) is more sensitive than T to chronic Leydig cell dysfunction. INSL3 may serve as a new marker for onset and progression of puberty [10]. After an initial rise in serum INSL3 concentrations at the onset of puberty, from around mid-puberty T and INSL3 levels decrease despite stimulation by increasing LH levels and remain in the low–normal range throughout puberty [11].

Different degrees of androgen deficiency can be observed in target tissues because the effects are not only dependent on T serum levels, but also on the individual's androgen sensitivity and on androgen receptor expression in tissues. KS boys displayed no evidence of diminished androgen action at a cellular level. Adequate androgen action is suggested by normal pubertal changes in serum SHBG and prostate-specific antigen (PSA). Serum PSA levels increase in KS boys as in

controls at puberty. Ejaculated volumes are normal in 55% of late pubertal KS patients *vs* in 78% of controls.

12.4 Spermatogenesis

Complete absence of germ cells is not always the axiom because even azoospermic KS patients occasionally present single foci of spermatogenesis in the testis [12] and spermatozoa have been found in ejaculate of KS adult in late puberty in a percentage from 2% to 8% [13].

Severe Sertoli dysfunction is revealed by decline in inhibin B (INHB) and anti-Mullerian hormone (AMH) and by rise in FSH.

After the initial phase of Sertoli cell proliferation during early puberty, dramatic changes occur in the tubules of the testis during mid-puberty. Sertoli cells regress in agreement with changes observed in serum levels of INHB. Stem cell apoptosis and fibro-hyalinosis of the tubular wall are established.

In KS, serum INHB levels during prepuberty are normal. The initial rise in INHB levels in KS and controls during early puberty can be reflected in an FSH-stimulated increase in INHB secretion by immature Sertoli cells. In early puberty, serum INHB levels in KS reflect the integrity of Sertoli cells. INHB concentration decreases very rapidly 12 months after the onset of puberty, reflecting the progressive degeneration of the gonadal tubules, and remains undetectable at the end of puberty in most patients with KS [14]. In the adult KS testes, virtually all germ cells and the majority of the Sertoli cells disappear, thus INHB remains undetectable [14].

Anti-Müllerian hormone (AMH) is expressed by prepubertal immature Sertoli cells and falls to low levels in puberty because it is negatively regulated by T from the time of puberty.

In KS boys at prepuberty and early puberty, AMH values are high. The physiological pubertal decline in serum AMH occurs later in KS boys [15]. The observed "delay" in pubertal decline in AMH in KS boys may be explained by the relatively low androgen secretion and thereby by impaired negative feedback.

From mid-puberty onward, FSH levels show a progressive increase with less pronounced rise in FSH in adolescents with spermatozoa than those without. LH rises above the normal range approximately 2 years after pubertal onset.

FSH increases later in puberty and its response to gonadotropin-releasing hormone (GnRH) stimulation becomes abnormal. The exaggerated response of FSH to luteinizing hormone-releasing hormone (LH-RH) suggests a progressive failure of the negative feedback mechanism related to the decreased INHB, due to the damage of the seminiferous tubules and subsequently of the Sertoli cells [16].

Activation of the hypothalamic pituitary testicular axis with an increase in T level seems to lead to degeneration of Sertoli cells. When the KS boys enter puberty, the testicles grow reaching a maximum volume of no more than 10 mL in patients aged 12–17 years and subsequently shrink down to less than 4 mL. In parallel to this, the gonadotropins rise to the greatly elevated levels. Major testicular histological alterations are seen that coincide with the activation of the pituitary gonadal

axis. The loss of germ cells accelerates through puberty, which leads to fibrosis and hyalinization of seminiferous tubules as well as hyperplasia of Leydig cells. The demise of germ cells and degeneration of Sertoli cells are accelerated markedly at the onset and during the puberty leading to Sertoli cell degeneration, Leydig cell hyperplasia, fibrosis, and hyalinization of the interstitium and peritubular connective tissue, as revealed by electron microscopy in KS testis. Thus, in boys with KS, after the initial phase of Sertoli cell proliferation, a subsequent phase of degeneration of Sertoli cells considerably increases during puberty [17].

The presence of only type A spermatogonia in 50% of KS testicular biopsies, performed during early puberty testis histology, suggests a maturative arrest in germ cell development.

It is possible that the molecular mechanisms induced by the altered dosage of X-encoded genes in testicular cells, like the androgen receptor gene, may accelerate loss of germ cells [18]. The alteration of development of testis in KS boys may be due to a defect in Sertoli and germ cell communication [19]. On the other hand, numerous report suggested that aneuploid germ cells sometimes can slip through the meiotic checkpoint and mature to spermatozoa [20].

Limited data exist regarding the optimal age to offer cryopreservation in KS patients, which is not determined. Sperm banking has been proposed when gonadotropins begin to rise [21].

To date, there is no agreement on the best age to perform sperm retrieval by testicular sperm extraction (TESE) in KS adolescents. For performing TESE, an age-dependent decline in retrieval rates cannot be estimated at present. The spermatozoa retrieval rate in KS adolescents varies between 0% and 70% in different small studies [22]. In patients with azoospermic nonmosaic 47,XXY Klinefelter, the difference in retrieval rate of spermatozoa is higher (62.5%), although not significant, in adult patients (25–39 years) than that (56%) found in younger age group (15–23 years) [23]. Regarding pubertal range, the retrieval rate of germ cells by TESE is very low for KS adolescents younger than 15 years (10%) compared with adolescents of 15 years and older (45%) [24]. To date, no prognostic markers or clinical parameters such as hormones and testicular volume or histology have been identified to predict the presence of spermatogenesis in males with KS. Mean testicular volume is not different between both sperm-negative and spermatozoa-positive patients. Only a combination of gonadotropin and T may have a predictive value as the presence of spermatozoa was unlikely in patients with T levels $\leq$7.0 nmol/L or LH levels >18 U/L [24]. On the basis of these data, TESE in puberty should be considered as an experimental procedure not able to guarantee future fertility.

12.5 Body Composition

In KS, an altered body composition with increased body fat and reduced muscle mass is reported. Metabolic syndrome (MS) is found in around 50% of KS men. A fivefold increased risk of developing the metabolic syndrome in adults with KS

compared to age-matched controls is reportedly found in a recent study where the strongest predictor of the metabolic syndrome was adiposity, especially truncal adiposity [25]. KS patients present an increased fat body mass, but normal lean body mass, despite normal T levels [26]. Reduced insulin sensitivity already found in pre- and peri-puberty could be a consequence of the altered muscle/fat ratio and could represent an initial marker of subsequent MS [27]. The features of metabolic syndrome appear to be independent of age or body mass index (BMI) and greater fat mass is already present before puberty indicating that genetic factors may also impact on body fat distribution.

KS patients should be considered at increased risk of developing MS and diabetes throughout their life; hence, it is imperative from an early age and during puberty to do a counseling regarding a healthy diet and regular exercise. Routine labs for metabolic evaluation, specially glycolipid profile, should be performed every 2 years in KS boys, even in ones with normal BMI, because lipid alterations and the presence of visceral fat are also found in more than a third of KS boys with normal BMI [28].

12.6 Bone Mass

When Klinefelter et al. [29] firstly described KS, osteoporosis had not been included in the clinical signs. Decreased bone mineral density (BMD) have been reported in adult KS patients with a substantial proportion (>40%) [30]. Reduced bone mass in KS has been usually ascribed to low T plasma levels [31].

The skeletal system undergoes a rapid growth between childhood and late adolescence. During puberty, testosterone and estrogen is fundamental for periosteal bone formation to reach adequate peak bone mass in the early twenties. Early onset of testosterone deficiency, which occurs frequently in KS adolescence, is estimated as a determinant risk factor for osteoporosis. However, the reduced bone mass does not appear to be always associated with low T levels and therefore osteoporosis can be also observed in normogonadic KS patients [32]. The bone alterations in KS men, such as the premature fusion, excessive calcification of coronal sutures, abnormal joint development and osteoarthritis [33], are not typically bone alteration observed in hypogonadal men.

Osteoporosis can be also found in KS patients with T levels in the normal range. Additional mechanisms could be involved in the pathogenesis of abnormal bone structure in this syndrome. The impaired KS bone structure and the bone loss might be related to other hypothetical genetic and hormonal factors. The pathogenesis of impaired bone mineral status may be multifactorial. High blood FSH levels influence bone through pro-inflammatory and pro-osteoclastogenic cytokines expression probably inducing bone loss [34].

Moreover, osteopenia of KS can also recognize an additional effect of genotype. X chromosome itself may lead abnormal bone structure as reported in a mouse model. The XXY mice has osteopenia due to trabecular bone changes, specifically a reduced trabecular thickness. The alterations in trabecular thickness appear to not

reduce bone strength as well as cause reduction in trabecular number [35]. The X-linked genes that escape inactivation, including Xq27, which is associated to BMD and area bone size, could be partly responsible for reductions in bone mass [36]. KS adolescents show reduced 25-hydroxyvitamin D, levels and higher parathyroid hormone (PTH) levels than controls even in absence of severe hypovitaminosis D.

The debate about whether testosterone replacement would increase the BMD in KS hypogonadal men is open. Previous study suggests that patients who do not achieve normal bone density during puberty are not able to achieve it later through androgen replacement treatment. T replacement should start early at puberty in KS men with a low serum T level in order to avoid bone mineral deficiency and to reduce the risk of future fractures [37].

The additional effect of genotype on osteopenia would provide a rationale for the difficulty in normalizing bone mass through androgen therapies in KS adult subjects [38]. For these reasons, several authors recommend to KS adolescents to consider adequate vitamin D supplementation and to maintain sex steroid levels in the normal range to prevent bone loss or improve low BMD [39].

12.7 Malignancies

Breast cancer, extragonadal germ cell tumors, and non-Hodgkin's lymphoma are the cancers found to be more prevalent in KS in the general population.

Breast cancer is 20–50 times more common in men with KS compared to men without KS with a prevalence of 3–7% but is very rare in adolescents [40]. The proposed responsible mechanisms are genetic predisposition, obesity with an impaired T/E2 ratio, and long-standing gynecomastia.

Patients with KS have also an increased risk for mediastinal and retroperitoneal germ cell tumors (GCTs). Extragonadal GCTs represent in KS a 65-times increased risk compared to the general population. Around half the reported cases of extragonadal GCT typically present with a peak age in adolescence. The presence of mediastinal GCT (M-GCT) in prepubertal boys could induce precocious puberty due to human chorionic gonadotropin (hCG) production, and in adolescents the most common symptoms are cough, dyspnea, or chest pain [41].

In boys with more than three sex chromosomes (48,XXYY), an association with non-Hodgkin's lymphoma was reported in a large British cohort [40].

12.8 Testosterone Supplementation

The correct management of adolescent males with KS remains controversial. The purposes of testosterone replacement treatment (TRT) for adolescents are to support linear growth and secondary sexual characteristics.

Conflicting evidences remain whether and when endocrine testicular dysfunction will decompensate during pubertal development.

One option is waiting until there is evidence of low or falling T and elevated LH together with presence of sign and symptoms of hypogonadism.

Another approach is to start low dose of T when an increase in gonadotropin levels outside the normal range for pubertal development occurs. A last approach would be to start TRT in early puberty before any evidence of gonadal insufficiency. The use of T in early puberty is indicated for the preservation of sperm retrieval for reproductive purposes later in life.

A personalized and tailored approach is required for each KS boy based on his natural pubertal development curve for a correct management of TRT.

Different formulations of T replacement have been applied in adolescence. Remaining endogenous production should not be suppressed, so treatment with short-acting T preparations should be preferred in puberty. Two formulations consistent in depot injections of testosterone and in topical testosterone gel are well tolerated by adolescent boys. Topical T is the suitable route of administration since it allows to reach adequate testosterone levels for the pubertal stage without the complete suppression of gonadotropins [42]. The goal is to maintain the serum T level at the upper end of the age-specific range. In addition to using total T, the best markers of the appropriate titration of androgen supplementation are the biological determinants such as pubic and axillary hair, increase in penile size, increase in semen volume, and nocturnal emissions. Androgen supplementation may prevent the development and persistence of gynecomastia, but cannot reverse gynecomastia [43].

Adolescents with KS show recurrently a decreased response to TRT when compared to an age-matched cohort, due to partial androgen resistance. For this reason, KS boys might require greater dose of TRT than controls [44]. Adverse effects from TRT can include development of pubic hair without progression of puberty, premature closure of epiphyses, aggressive behavior, and exacerbation of acne.

TRT has previously been reported to be a negative influence on future fertility of KS patients, based on low sperm retrieval rate within a small series of study population of KS. More recently, two studies in adolescents and young men with KS have yielded good results as documented by retrieval spermatozoa rate respectively of 70% and 52% [22, 23].

Therefore, previous replacement treatment with moderate doses of T is not likely to have a deleterious impact on the recovery of sperm cells by TESE. Some authors suggest that testosterone treatment and possibly adjuvant aromatase inhibitors should be started in early puberty before the first signs of declining Sertoli cell function (rising FSH and LH levels) in order to preserve testicular tissue before hyalinization occurs and to enhance sperm retrieval success rates for men with KS [22]. Current evidence does not justify postponing the initiation of TRT in adolescent boys with KS to address issues of reduction in their future fertility although larger studies are necessary to confirm these findings.

12.9 Conclusions

The medical management of KS during transition should require a multidisciplinary team to deal with all the aspects of the syndrome. Early detection of this syndrome is recommended in order to offer treatment and intervention at the appropriate ages and stages of development in order to improve the quality of life and to decrease the morbidity of the patients in the long term. It is finally important to have a close interaction with parents, because the KS boys have poor perception of the negative repercussions of medical noncompliance. Clinic and laboratory periodic follow-ups are decisive to reach treatment goals.

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Genetic of Gametes

13

Marica Franzago and Liborio Stuppia

13.1 Background

Klinefelter syndrome (KS), characterized by the presence of a 47,XXY karyotype, is the most common chromosome aberration in males, affecting from 1/500 to 1/700 men in general population [1]. Although the clinical features and the chromosomal pattern associated with this condition were detected a long time ago [2, 3], so far, the pathogenic mechanisms underlying KS have not been fully elucidated yet. Infertility with small testicles and tall stature are constant findings in KS patients, while other symptoms, such as less body hair, gynecomastia or other female secondary sex traits, can be detected only in a portion of patients. For this reason, despite its high prevalence in the population, this condition remains undiagnosed in many cases and seek for medical care usually occurs as a consequence of infertility of the couple.

A specific pathogenic mechanism in KS phenotype is known only as concerning high stature, due to the presence of three copies of the short stature homeobox-containing gene (*SHOX* gene), mapped within the pseudo-autosomal region 1 (*PAR1*) of the sex chromosomes. On the other hand, less information is available

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about the specific mechanisms underlying spermatogenesis failure and the other clinical signs.

In order to understand the bases of alteration of spermatogenesis in KS, it is of crucial importance to have a clear picture of the normal process of gametogenesis in human males.

13.2 Male Gametogenesis

The human gametogenesis is a highly complex, dynamic and well-organized process in which specialized reproductive haploid cells are produced from the gonadal primordial germ cells (PGCs).

The spermatogenesis, in which spermatozoa are produced from spermatogonial stem cells, is a complex process characterized by the reduction in the chromosome number to haploid pattern as well as by chromatin remodelling and epigenetic changes. These latter modifications occur in the last phase of spermatogenesis, known as spermiogenesis, which requires the transition from the spermatid to the sperm. In this passage, the nucleus histones are replaced by the protamine (small molecules rich in arginine), leading to the chromatin condensation and inducing sperm deoxyribonucleic acid (DNA) compaction. After fertilization, before the pronuclear formation, the protamine-to-histone transition occurs leading to chromatin decondensation, and the maternally derived histones replace the sperm protamines.

Epigenetic modifications, including DNA methylation, H3K27-H3K4 and H3K9 methylation, work cooperatively to regulate phase-specific gene expression and are essential for the formation of mature sperm and early embryo development [4].

13.2.1 Spermatogenesis Disruption in KS

Although KS is clinically characterized by a highly variable phenotype, infertility represents a constant condition in patients affected by this disorder. In the large majority of cases, KS patients show non-obstructive azoospermia, and only in rare cases, motile sperms can be detected in their ejaculate [5]. This dramatic clinical feature is the result of a progressive degeneration of testicular function in KS patients, the normal testicular architecture being replaced by tubular atrophy and sclerosis, with maturation arrest and degeneration to fibrosis and hyalinized tissue. A crucial point in this issue is the identification of the period in which this process of degeneration begins. In fact, although pre-pubertal testicular development in KS is generally described as similar to the one of boys with normal karyotype, early studies carried out in foetal testes had suggested the presence of reduced numbers of germ cells even between 18 and 22 weeks of gestation. However, data collected by different groups were discordant on this specific point [6, 7]. Also, Leydig cells have been suggested to be involved in testis dysregulation, since low levels of serum testosterone are detectable during the first months of life in non-mosaic 47,XXY patients [8]. On the other hand, Sertoli cells appear to be histologically intact in both the foetal and neonatal periods. This subtle alteration in foetal and

neonatal testes likely represents the basis for the later testicular failure occurring during puberty when a rapid deterioration in the production of germ cells and in the histological picture of KS testis become evident [9]. This degeneration is probably accelerated by stimulation of gonadal tissue by the hypothalamic-pituitary-gonadal (HPG) axis, via the inactivation of apoptosis-related genes within the spermatogonial cell line as spermatogonia differentiate into primary spermatocytes and progress through meiosis [10].

More recently, it has been again suggested that, despite the supposedly normal pre-pubertal testicular development, the foetal KS testis actually displays impairment of foetal gonocyte differentiation, with loss of prespermatogonia [11]. Details about this novel acquisition will be discussed in the paragraph analysing testis transcriptome in KS.

The large majority of data collected about testis dysfunction in KS patients concerns cases with full 47,XXY karyotype. However, about 15–20% of KS patients show a mosaic pattern, usually involving two cell lines (46,XY/47,XXY) or, in a very limited number of cases, more cell lines (e.g. 46,XY/47,XXY/48,XXXY) [12]. The mosaicism occurs: (1) from non-disjunction in an early mitotic division of the developing 46,XY zygote; or (2) from loss of one X-chromosome of a 47,XXY conception due to anaphase lagging. The KS patients with mosaicism generally are less severely affected [1, 13], while the phenotype progressively worsens with the polysomy severity (e.g. 49,XXXXY) [1, 14]. The low-grade mosaicism, due to the presence of a few cells with normal karyotype, may be related to some germ cell preservation [15]. Thus, whereas most KS men are azoospermic and require assisted reproductive technology (ART) based on testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI) techniques to generate a child, the mosaic KS patients are characterized by less severe infertility phenotypes with severe oligozoospermia or residual single foci with preserved spermatogenesis in the testis [16]. In these cases, sperms to be used in ART can be collected within the ejaculate [9].

Literature data report high sperm retrieval rates and ensuing success rates in KS patients [17, 18]. Nevertheless, the risk of transmission of chromosomal aneuploidies to the offspring of KS males should be taken into account. Two hypotheses to clarify the high aneuploidy frequency in KS sperm have been suggested: (1) 47,XXY spermatogonia can undergo meiosis and produce aneuploid spermatozoa [19, 20]; and (2) the XY spermatogonial cells, due to the compromised XXY testicular environment, are particularly susceptible to a variety of meiotic abnormalities, including non-disjunction of the sex chromosomes [21].

13.3 Gene Expression Alterations in KS Testis

Useful information about KS testis dysregulation comes from studies investigating ribonucleic acid (RNA) expression profiles in KS patients as compared to normal controls. D'Aurora et al. [22], using a transcriptome analysis by expression microarrays on testis biopsies of azoospermic KS patients and normal controls, evidenced a differential up- and down-expression of 656 and 247 transcripts, respectively.

Interestingly, the large majority of the deregulated transcripts were expressed by Sertoli cells and Leydig cells. Functional analysis of the transcripts involved in these modifications evidenced changes in the expression of genes involved in cell death, inflammatory response, lipid metabolism, steroidogenesis and blood-testis-barrier formation and maintenance. Based on these results, the authors suggested that 47,XXY karyotype is associated with alterations in the modulation of 100 genes in the somatic components of KS patient testis. The increased Leydig cell steroidogenic function, together with the impairment of inflammatory pathways and blood-testis-barrier structure, is likely related to an increase in apoptosis in KS testis.

The same group subsequently performed an additional study on testis biopsies of KS patients with hypospermatogenesis, a feature present in a minority of subjects with 47,XXY karyotype. In this case, KS testis showed a differential up- and down-regulation of 303 and 747 transcripts, respectively, as compared to controls. Unlike azoospermic, oligozoospermic KS patients showed in their testis a majority of down-regulated transcripts involved in spermiogenesis failure and testis morphological defects, whereas up-regulated genes were responsible for testis apoptotic processes. Functional analysis of the transcriptionally altered genes indicated a deregulation in cell death, germ cell function and morphology as well as blood-testis-barrier maintenance and Leydig cell activity. Taken together, these data lead authors to suggest a complex scenario with spermatogenic impairment as the consequence of functional and morphological alterations in both germinal and somatic components of KS testis [23].

Other studies, carried out by RNA-sequencing, evidenced the presence of 235 differentially expressed transcripts (DETs) in the adult KS testis, with enrichment of long non-coding RNAs (lncRNAs), but surprisingly, not of X-chromosomal transcripts [11]. When the authors compared the DETs in foetal, pre-pubertal and adult KS testes, the overlap was limited, probably due to different mechanisms responsible for the testicular architecture and cellular composition at the different developmental stages [11].

More recently, a significant over-expression of lncRNA GAS5 in peripheral blood of non-mosaic KS patients compared to controls was evidenced. The lncRNA GAS5 seems to be involved in several biologic processes, including inflammatory and autoimmune diseases, vascular endothelial cell apoptosis and atherosclerosis, as well as cellular growth and proliferation, cellular development and cell-to-cell signalling [24]. Further investigations are necessary to understand whether the lncRNA GAS5 over-expression is involved also in the pathogenesis of testis damage in KS patients.

13.4 Epigenetics and KS

Epigenetic alterations may play a role in the biological mechanisms underlying the clinical KS phenotype by affecting chromatin structure and gene expression. Few studies provided evidence that KS may be associated with widespread changes in the methylome of blood and brain tissue [25–27]. Unfortunately, these studies were either performed on small KS cohorts or used arrays with a small number of CpG

sites; in addition, only one investigated the correlation between changes in the methylome and alterations in the transcriptome on brain tissue of a single post-mortem KS patient compared to male and female controls [25].

Recently, Skakkebæk et al. [28] performed both genome-wide DNA methylation profiling of leucocytes from peripheral blood samples from 67 KS patients, 67 male and 33 female controls, and genome-wide RNA-sequencing profiling in a subset of 9 KS patients, 9 male and 13 female controls. The authors identified a unique epigenetic and genetic landscape of both autosomal chromosomes and the X-chromosome in KS. In particular, in agreement with earlier studies [25–27], KS was mainly associated with hypermethylation and to a lesser extent hypomethylation. Concerning autosomal differentially methylated genes, the study of Skakkebæk et al. [28] compared to the one of Sharma et al. [26] showed an overlap among four genes, all hypermethylated, whereas no overlap was detected among X-chromosomal genes.

Furthermore, it has been evidenced that the X-chromosome inactivation mechanism in KS, to some extent, is comparable to the one seen in women, although with an X-chromosomal gene expression profile more similar to 46,XY males, pointing out new candidate genes, which may likely be involved in the KS phenotype [28].

13.5 KS and ART

Infertility in KS males has been overcome by utilizing sperm retrieval methods, such as conventional testicular sperm extraction (c-TESE) and microsurgical TESE (micro-TESE), followed by ICSI [29]. In fact, an average sperm retrieval rate (SRR) of 50% by c-TESE and by micro-TESE in men with KS has been reported and live children could be obtained in approximately 16% of KS males who undergo TESE approach [5, 18]. Although the specific predictors of underlying success of this approach are still conflicting, it has been found that age at ART treatment (below 35 years) ensures a better chance of positive TESE [30–36]. In contrast to this view, in the studies analysed in a meta-analysis, the successful sperm retrieval rates in KS is independent of age [18].

The potential risk of chromosomal aneuploidy in resultant offspring, following increased incidence of genetically imbalanced sperm [37], is a concern for KS males. Although the data of literature are conflicting, to overcome this important issue, it has been suggested that couples with non-mosaic KS should undergo pre-implantation genetic testing (PGT) of embryos and fluorescent in-situ hybridization (FISH) analysis of sperm [37].

13.6 Conclusions

Although the chromosomal basis of KS was identified since 1958, so far, the pathogenesis of testicular damage and consequent infertility in these patients have not been identified yet. Useful information has been provided by studies analysing testis transcriptome of KS patients as well as the role played by epigenetic modifications

in the pathogenesis of the different clinical signs of this condition. However, the most important issue in this topic is represent by the evidence that KS patients, even in the non-mosaic pattern, are not irremediably infertile. The use of ART protocols based on TESE and ICSI can allow a significant portion of these patients the opportunity to generate a child. On the other hand, the risk of sex chromosomal aneuploidies must be always considered. Further opportunities for KS patients in the future would likely be provided by increasing strategies aimed at early diagnosis and novel treatments of this condition.

Due to the high prevalence of 47,XXY karyotype in the population and the possibility to detect this chromosomal abnormality by using molecular tools that are less expensive and less time consuming as compared to cytogenetic investigation, the possibility of neonatal screening of KS should be considered within the programme of public health strategies aimed to improve the reproductive fitness of the population.

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Phenotype of the Adulthood

14

Sara De Vincentis and Vincenzo Rochira

14.1 Introduction

The main clinical features of Klinefelter's syndrome have been well defined since the first description of the disease. In 1942, Klinefelter, Reifenstein, and Albright reported nine adult males with a syndrome characterized by gynecomastia, small and firm testes, azoospermia and elevated serum levels of follicle-stimulating hormone [1]. The etiology of this syndrome remained unknown until 1959, when the presence of an extra X chromosome in the karyotype of patients with Klinefelter's syndrome (genotype XXY) was demonstrated by Jacobs and Strong [2]. The supernumerary X chromosome has a pivotal role in determining the major findings of Klinefelter men [3]. However, the clinical picture described above has been characterized only on the basis of a small number of affected patients, precisely those seeking medical consultation and probably displaying the more severe degree of clinical features [4]. Less severe or mild forms have also been documented and most of them remain often undiagnosed, because of the poor symptomatic manifestations [4–6]. Thus, our knowledge about signs and symptoms of Klinefelter's syndrome and their frequency is still limited since most of the patients remain overlooked.

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14.2 The Heterogeneity of Klinefelter's Syndrome Phenotypes

It would be almost simplistic straitening the clinical presentation of adult men with Klinefelter's syndrome to the traditional phenotype first described in 1942, since the classical findings are often missing. Indeed, there is considerable phenotypic variation among men with Klinefelter's syndrome [4]. Alternative phenotypes have been recognized, in which patients present with fewer and/or less evident clinical features than observed in the classical phenotype [6, 7]. From a clinical standpoint, the appearance of men with a 47,XXY karyotype may vary from mild to severe forms. The broad spectrum of phenotypes in Klinefelter's syndrome depends on many factors such as patient's age, genetic background, degree of hypogonadism and delay in the diagnosis [4, 8] (Fig. 14.1).

The high frequency of these less severe or mild forms characterized by poor symptomatic manifestations explains, at least in part, why most of the patients with Klinefelter's syndrome remain undiagnosed [4, 9]. It has been estimated that 25–40% of men with Klinefelter's syndrome are identified [10], but this esteem

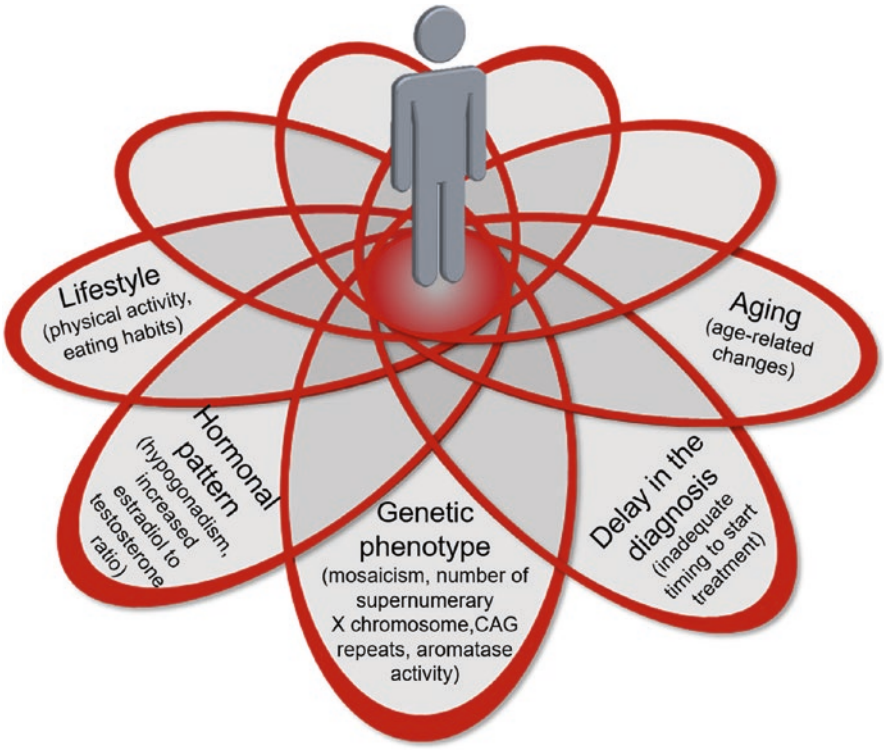

Fig. 14.1 The broad spectrum of clinical phenotypes in Klinefelter's syndrome depends on the combination of several genetic and non-genetic factors

needs to be further confirmed since data on both prevalence and missed diagnosis range widely according to different studies [11]. Moreover, signs and symptoms are rarely simultaneously present in the same patient, thus leaving the diagnosis challenging and further overlooked and delayed. A prompt clinical management, including the start of testosterone replacement therapy in case of hypogonadism, and lifestyle modifications can partly prevent long-term consequences in terms of worsening of the phenotype [4, 11] and the occurrence of comorbidities [12, 13].

14.3 Pathogenesis of Klinefelter Phenotype

The Klinefelter phenotype is the result of the combination of several factors that all contribute to determine the appearance and severity of symptoms and signs (Fig. 14.1). Relative androgen deficiency in Klinefelter's syndrome is not responsible for all the clinical features. Another factor that is mainly involved in phenotype determination is the supernumerary X chromosome causing overexpression of certain X-bound genes [4, 5, 14]. Overall, the severity of the phenotype is strongly related to the severity of expression of genetic defect, androgen deficiency, and androgen receptor sensitivity [8]. Less severe forms of genetic abnormalities generally result in less severe clinical features. For example, Klinefelter patients with mosaicism show few clinical signs and symptoms and less severe endocrine abnormalities than their non-mosaic counterparts [15]. Conversely, the phenotype progressively worsens with the severity of polysomy (e.g., 49, XXXXY) [4–6]. Furthermore, the profile of methylation seems to be modified in the DNA of patients with Klinefelter's syndrome [11]. Large areas of hypermethylation (more frequently) together with areas of hypomethylation (less frequently) are found in the DNA and they may account for the high degree of variability of the phenotype of these patients ascribed to genetic determinants [11, 16, 17].

Thus, phenotypic features of Klinefelter syndrome can be distinguished in androgen-dependent and supernumerary X-dependent signs, according main underlying pathogenetic mechanisms. Unraveling signs and symptoms due to androgen deficiency from those due to chromosome abnormalities are crucial in order to improve the outcome of testosterone replacement treatment, to establish how the disease should be monitored during the follow-up, and to inform the patient on what the expected results are (Table 14.1).

Other genetic aspects may influence the phenotype. Independently from circulating testosterone, the length of CAG trinucleotide repeats within the androgen receptor may modulate the receptor activity and, thus, sensitivity to testosterone in males [18] (Fig. 14.1). In the last decade, the CAG length was supposed to be associated with the phenotypic variability even in Klinefelter's syndrome: a longer CAG seems to be positively correlated with the development of gynecomastia, small testes and above-average height [8, 14]. Similarly, even genetically determined differences in the aromatase expression and activity [19] may influence the endocrine phenotype in Klinefelter patients [20, 21] (Fig. 14.1).

14.4 Clinical Presentation and Phenotype

The "prototypic" adult man with Klinefelter's syndrome has traditionally been characterized as tall, with gynecomastia, small testes, azoospermia, sparse body hair, narrow shoulders, and broad hips [1] (Table 14.1).

In adulthood the very low testicular volume and firm consistency of the testes represent the most important clinical findings [4–6]. Testicular size can be assessed by palpation with the help of testis-shaped models of defined sizes (e.g., Prader orchidometer) or more precisely with ultrasonography. The discrepancy between testes growth and male physical appearance starts to be evident at puberty when sexual secondary characteristics and penile growth progress towards the adult phenotype but the enlargement of the testes remains blocked to testicular size typical of infancy [4, 22]. The gap between testicular growth and the completion of pubertal development of the penis and secondary sexual characteristics is extremely useful to prompt the diagnosis of Klinefelter's syndrome during the first visit in both adult men and pubertal boys [4, 22]. Notwithstanding the completion of pubertal development, in fact, the volume of each testis as well as the bi-testicular volume are significantly lower than in non-Klinefelter men, being below 10 mL (5 mL for each testicle) [5].

Considering anthropometry, Klinefelter patients are taller when compared to controls [23, 24]. Moreover, the increased leg length exceeds that of the other segments of the skeleton, thus contributing to both final tall stature and eunuchoid proportions of the body [5, 23, 24] (Table 14.1). Of note, the arm span seldom exceeds the body height in men with Klinefelter's syndrome, differently from other forms of hypogonadism [4]. This peculiarity cannot be ascribed only to hypoandrogenism, but rather to the existence of an extra copy of the SHOX gene, mapping

Table 14.1 Phenotypic features and comorbidities of men with Klinefelter's syndrome grouped according to their putative reversibility

	Modifiable features	Partially modifiable features	Unmodifiable features
Signs	Sparse body and facial hair Female pubic escutcheon Weight, BMI, WC, WHR Reduced muscle mass Bilateral gynecomastia Impaired estradiol/testosterone ratio		Longer legs Small testes Eunchoid habitus Tall stature Elevated gonadotropins
Symptoms	Impaired sexual desire Erectile dysfunction Weakness and loss of vigor Impaired well-being Impaired QoL		Azoospermia/ infertility
Comorbidities	Metabolic abnormalities (overweight, obesity) Mood disturbances	Reduced bone mineral density	

BMI body mass index, *WC* waist circumference, *WHR* waist-to-hip ratio

to X and Y chromosomes and involved in linear growth [5, 24–26]. This may also explain why the increased height is present still during infancy, before pubertal onset [4, 5].

Waist circumference, body mass index (BMI) and waist-to-hip ratio are significantly greater in men with Klinefelter's syndrome than in controls [12, 23], but the physical trait may vary from slim to normal and obese phenotype depending on individual differences, ethnicity [27] and age.

The degree of virilization widely differs, with a progressive worsening with advancing age. Some patients develop an overt hypogonadism, with evident signs (horizontal pubic line, scant body, axillary, facial hair, and poor muscle mass) and symptoms of under-virilization [1, 5, 25] (Table 14.1). After the age of 25, up to 70% of men with Klinefelter's syndrome complain of symptoms related to an overt hypogonadism (decreased libido, erectile dysfunction), but this percentage varies according to different studies (see the subsequent paragraph for further details) [28] (Table 14.1). The main symptom that usually induces the adult patient to seek medical consultation is infertility. Most patients affected by Klinefelter's syndrome, in fact, are infertile because of azoospermia (Table 14.1). Testicular histopathology displays various patterns ranging from the classical and most severe (germ cell aplasia, total tubular atrophy or hyalinizing fibrosis and relative hyperplasia of Leydig cells) to a less severe pattern in which foci of spermatogenesis up to the stage of mature testicular sperm can be detected [29].

As Klinefelter patients progress to adulthood, hypogonadism and related conditions tend to become more prevalent, contributing to the clinical appearance of patients (see the subsequent paragraph for details). Overweight, obesity and a gynoid fat distribution are common findings in Klinefelter patients, as deepened in the paragraph below [13, 23] (Table 14.1).

Bilateral gynecomastia of varying degrees is present in nearly half of patients [1, 25, 30], with no higher risk of developing mammary carcinoma than in normal men [25, 31]. Decreased testosterone levels together with relatively increased estradiol levels have been initially proposed as a possible explanation of gynecomastia [25]. However, the sex steroid hormonal milieu in Klinefelter patients, especially the role of hyper-estrogen status, is still quite controversial [21, 32]. Testosterone treatment can marginally improve the regression of gynecomastia; otherwise, a surgical resolution by means of mastectomy and cosmetic breast surgery should be suggested, especially if gynecomastia troubles the patient [25, 32].

Even though rare, an increased risk of congenital malformations has been found in patients with Klinefelter's syndrome [3, 33]. Among urogenital malformations, inguinal hernia, alteration of testicular descent (from cryptorchidism to testis retention), the latter ranging from 6% to 27% [25], seem to be more frequent in boys with Klinefelter's syndrome than controls [5, 6, 34]. The easiness of detection at physical examination of genitalia can explain the elevated prevalence of urogenital malformatins compared to other malformations more difficult to be identified.

Intellectual abilities are not impaired, but deficits in specific domains of cognition (e.g., reduction in speech and in language abilities, verbal processing speed) may be present [11, 35]. Besides, the old concept of a strong association among

Klinefelter's syndrome and criminal behavior, psychiatric disorders, and mental retardation is now considered outdated, since no evidence-based data have subsequently confirmed this erroneous long-held view [5, 6, 25].

14.5 The Endocrine Phenotype in Klinefelter's Syndrome

At the beginning, the finding of hypergonadotropic hypogonadism in men with Klinefelter's syndrome led to the concept that serum testosterone below the normal range was a feature constantly associated with this syndrome [1]. However, more recent studies pointed out the finding of normal serum testosterone in a consistent percentage of cases at puberty and in adulthood. Different case-control studies reported mean testosterone levels within the normal range in Klinefelter patients, but significantly lower compared to age-matched non-Klinefelter men [20, 21]. Even though the majority of studies found lower serum total testosterone in men with Klinefelter's syndrome compared to controls [21], few studies did not find any difference [12, 14, 36, 37]. Actually, the prevalence of hypogonadism in Klinefelter patients widely varies in literature and it has not been defined with precision so far.

Furthermore, Klinefelter's syndrome was traditionally considered to be associated with serum estradiol levels higher than the normal male range by virtue of the association of the syndrome to the gynecomastia and some other female features (e.g., female pubic escutcheon, sparse facial and body hair) [1–4, 6, 20, 21]. Subsequent studies have shown that the amount of circulating estrogens does not differ from that of non-Klinefelter men [5] but the estradiol to testosterone ratio is significantly increased in men with Klinefelter's syndrome compared to age-matched controls [21]. An individual's genetically determined predisposition to increased aromatase activity and expression may result from some genetic variants as it happens in men not affected by Klinefelter's syndrome [19]. Thus, an increased aromatase activity may worsen hypogonadism due to raised conversion of testosterone in estradiol resulting in a further reduction of serum testosterone.

Finally, it is important to take into account that other comorbidities, not strictly related to Klinefelter's syndrome, can contribute to the patients' clinical appearance masking some of the specific aspects described above [38]. Particularly, hyperandrogenism due to other diseases, such as adrenal congenital hyperplasia, may counterbalance the hypogonadism induced by Klinefelter's syndrome.

14.6 Phenotype and Comorbidities

The phenotype depends at least in part from the associated comorbidities that may develop during time. Even though the comorbidities are object of other chapters of this book, here we will summarize their contribution to the determination of inter-individual differences of the phenotype.

Men with Klinefelter's syndrome may display overweight and obesity, but there is a high degree of variability in the anthropometric features of Klinefelter men ranging from underweight to normal weight, overweight and obesity [11] (Table 14.1). It is clear that lifestyle and eating behavior are determinant for the development of overweight, but several studies suggest that the syndrome per se may also predispose to fat accumulation especially at the visceral site [34]. Hypogonadism plays an important role on body composition changes, and testosterone therapy may revert in part overweight/obesity [39, 40]. However, it is not clear what came first, hypogonadism or overweight due to the bidirectional relationship between low circulating testosterone and fat mass accumulation.

Even the metabolic syndrome, insulin resistance and type 2 diabetes mellitus—all of them are strictly related to overweight/obesity and increased visceral fat—seem to be more prevalent in men with Klinefelter's syndrome than in healthy matched men [11–13, 41]. However, possible differences concerning overweight/obesity and the metabolic profile among patients living in different countries with different lifestyle, ethnicity and diet remain to be elucidated.

Bone mineral density may be decreased resulting often in osteopenia or less frequently in overt osteoporosis; in addition, reduced muscle strength may develop [5, 25, 42] (Table 14.1).

Finally, there is no evidence of an increased risk of thyroid dysfunctions in Klinefelter's syndrome [43].

For details about comorbidities in Klinefelter's syndrome, see specific chapters.

14.7 The Role of Aging on the Clinical Phenotype

Aging is associated to several changes, including endocrine modifications in the general population [44]. Thus, aging has a crucial role in influencing the phenotype even in men with Klinefelter's syndrome.

Testosterone levels decline in serum with advancing age in normal men [45]. The same trend is seen in men with Klinefelter's syndrome with normal testosterone in early adulthood declining at a younger age than in the general population [46], suggesting premature gonadal failure. Accordingly, serum testosterone that is often in the normal range in early adulthood progressively reaches values below the normal range in 65–85% of patients with Klinefelter's syndrome after the age of 25 [25, 28, 47]. Thus, in front of normal serum testosterone in a Klinefelter young man this should be periodically checked in order to promptly diagnose hypogonadism few years later.

Similarly, all the parameters that indirectly account for changes in body composition and the increase of adiposity result correlated with age in patients with Klinefelter's syndrome as it happens for the normal population [12]. Thus, it is clear that aging plays an important role on the worsening of the metabolic phenotype in men with Klinefelter's syndrome independently from their predisposition to develop overweight/obesity and the metabolic syndrome [12].

14.8 The Impact of Clinical Phenotype on Quality of Life (QoL)

The degree of clinical phenotype may also impact QoL of patients with Klinefelter's syndrome. Body appearance and all the aspects related to body composition (overweight/obesity) and overall the presence and the degree of gynecomastia may influence the self-perception of the patient's body image (especially gynecomastia) and may result in psychological, gender-identity, self-confidence, self-esteem and social (relationship troubles) complications finally leading to reduced QoL [48]. Similarly, learning disabilities, mood (reduced social interaction) and the awareness of having a chromosomal defect may affect almost all the above-mentioned psychosocial issues. Furthermore, infertility leads to troubles related to the daily patient's life, which are linked to the poor probability of fatherhood and related problems in the relationship with the partner. One of the most considerable issues for a patient with Klinefelter's syndrome concerns the timing (when) and the modality (how) to communicate to the partner that he is a carrier of the syndrome and that the syndrome itself implicates a high probability to be not able to conceive children. Recently, a study investigating the literature and results on daily experience of men living with the syndrome concluded that the severity of phenotype influences the outcomes concerning psychosocial variables and QoL and that these patients suffer from feeling themselves as different from the general population [49]. In conclusion, the clinical phenotype may be related to psychosocial problems and may result in anxiety, depression and sleep disorders.

Klinefelter and Clinical Phenotype—The Practical Clinical Corner

Klinefelter patients are traditionally described with eunuchoid skeletal proportions, characterized by increased tallness, mainly related to increased leg length. Often, overweight and obesity are part of the phenotype.

Practical issues:

- Physical examination of Klinefelter patients should include the following anthropometric measurements: height, weight, waist and hip circumferences, sitting height and arm span.
- The evaluation of body proportions should be completed through the calculation of upper-to-lower segment ratio, span-to-height ratio, body mass index (BMI) and waist-to-hip ratio.

Small and firm testicles represent the main clinical hallmark that may be easily revealed by testicular examination (size and consistency). Some congenital anomalies of genitalia have been reported.

Practical issues:

- An accurate exploration of external genitalia should be performed. Scrotal palpation coupled with the evaluation of the volume of the testes by the Prader orchidometer is mandatory.

Gynecomastia and hypogonadism are highly prevalent.
Practical Issues:

- Gynecomastia should be explored by observation and palpation.
- The distribution of body and facial hair should be evaluated.
- The interview should include sexual issues: sexual desire impairment and data on erectile function.
- Symptoms and signs (reduction in height, back pain, physical examination of the spine) related to reduced bone mineral density should be checked.

14.9 Conclusions

Clinical phenotype of Klinefelter's syndrome has been partially disclosed and still needs to be fully elucidated. Accordingly, many cases of Klinefelter's syndrome remain undiagnosed due to the substantial variation in clinical presentation. Clinicians should be aware of the broad spectrum of clinical phenotypes performing an accurate physical examination in case of a suspected diagnosis. Besides, the clinical phenotype changes according to different period of age and physicians should consider these modifications in order to perform physical examination and clinical synopsis in a tailored fashion. Early recognition and prompt clinical management can improve quality of life and prevent disease-related consequences in these patients.

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Hypothalamic–Pituitary Axis Function

15

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15.1 Physiology of the Hypothalamic–Pituitary Axis

The hypothalamus and pituitary gland have to be considered a unique functional unit, given the tight anatomical connection, and the finely tuned functional modulation. Virtually, all physiological functions are directly or indirectly regulated by the hypothalamus, which releases regulatory factors acting upon target cells of the pituitary and stimulating the secretion of hormones controlling the endocrine glands of human body. The median eminence (ME), a highly specialized anatomical arrangement of hypothalamic ventral bulge, from which the pituitary stalk originates, releases neurosecretion into the pituitary portal blood system [1]. The pituitary gland comprises two lobes: the posterior lobe, or neurohypophysis, and the anterior lobe, or adenohypophysis. The neurohypophysis is constituted by the terminal part of the axons of oxytocin- and vasopressin-secreting cells, the magnocellular neurons, which are located in the hypothalamic supraoptic and paraventricular nuclei, and is deputed to the control of normal delivery and lactation, and to the maintenance of body fluid balance, and modulation of vasoconstriction to increase blood pressure [2]. The adenohypophysis is composed of the pars distalis, the pars intermedia, and pars tuberalis. In the adenohypophysis, different cell types can be

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distinguished, which are responsive to different hypothalamic releasing factors and control the function of different endocrine organs: the gonadotropic cells, which are responsive to gonadotropin-releasing hormone (GnRH), secrete gonadotropins, namely, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), regulating gonadal function; the thyrotropic cells, which are responsive to thyrotropin-releasing hormone (TRH), secrete the thyroid-stimulating hormone (TSH); the lactotropic cells, which are responsive to TRH and additional hypothalamic polypeptides, secrete prolactin (PRL); the corticotropic cells, which are responsive to corticotropin-releasing hormone (CRH), secrete adrenocorticotropic hormone (ACTH); and the somatotropic cells, which are responsive to growth hormone-releasing hormone (GHRH), secrete growth hormone (GH) [3].

15.1.1 Gonadotropic Axis

GnRH-producing cells are located in the preoptic area of the brain in most mammals, and project into the ME, where GnRH is stored in neurosecretory terminals; GnRH secretion depends on the stimulation by kisspeptin through the activation of its GPR54 receptor, located on the surface of the GnRH neurons [4]. Once GnRH is released into the ME, it reaches the pituitary, where it stimulates the release of the gonadotropins LH and FSH. Since GnRH secretion is pulsatile, gonadotropins release also occurs in discrete peaks, more evident for LH, due to the shorter half-life in the blood stream, compared to FSH [3]. The modulation of GnRH pulses' frequency determines the preferential release of LH or FSH [3]. The generation of GnRH pulses requires a coordinated release from multiple neurons, through the synchronization of GnRH neuron population excitation [4]. GnRH neurons projections, or dendrons, simultaneously receive and integrate synaptic inputs, since they possess both axonal and dendritic characteristics, before finally acquiring an axonal morphology within the ME and ramifying into numerous terminals, by reaching blood vessels' endothelial cells; therefore, dendrons might be an ideal location for putative afferent axons to modulate the excitability of multiple GnRH neuron dendrites, and for multiple GnRH neurons to align their firing pattern [5]. Moreover, endothelial cells in the ME might modulate GnRH release through the pulsatile and cyclic pattern of secretion of nitric oxide (NO), whereas in the GnRH neuron cell body, basal NO synthase activity might provide the tonic inhibition of the GnRH neural system that is required to maintain nadir levels of gonadotropins [5]. Overall, the intricate relationships between pulsatile GnRH release, secretory competency of the pituitary gonadotropic cells, and regulatory mechanisms within the vasculature, generate the rhythmic fluctuations in LH and FSH secretion; indeed, high frequencies (>1 pulse per hour) of GnRH pulses stimulate LH production, whereas low frequencies (<1 pulse per 2–3 hours) of GnRH pulses preferentially induce FSH production [5]. FSH and LH are required to maintain proper spermatogenesis and sex hormones production, by stimulating Sertoli cells and Leydig cells function, respectively, within the testes [6]. Anti-Müllerian hormone (AMH) is produced by fetal Sertoli cells and is responsible for the regression of the Müllerian ducts in the male fetus, at the time of sexual differentiation. In healthy boys, there is a steep increase in circulating AMH concentrations during the first months of life, in the so-called

mini-puberty, followed by a decline to a relatively stable level until the time of puberty, when a renewed decline to a further decreased stable level through adolescence and adulthood is found [7]. Insulin-like 3 (INSL3) is a marker of Leydig cell differentiation, playing a role in testicular descent during fetal development, by acting on gubernaculum, and is also a marker of male puberty onset [8]. INSL3 is secreted by Leydig cells in response to LH and is reduced in situations of undifferentiated or altered Leydig cell status, thereby representing a good marker of Leydig cell function. Since INSL3 receptors are found on germ cells, a local role for INSL3 during spermatogenesis is assumed, supported by evidences of lower circulating INSL3 levels in azoospermic men and a weak correlation with sperm number; nevertheless, more data are required to firmly corroborate these evidences, to support a role as a local regulator of human spermatogenesis [7]. Pituitary function is also regulated directly, and through the hypothalamus, by gonadal hormones. Testosterone and dihydrotestosterone at physiological levels exert a negative feedback mechanism acting mainly on the hypothalamus, by decreasing the frequency of GnRH pulsatility, whereas estrogens reduce the amplitude of LH and FSH peaks at the pituitary level [7]. Apparently, much of the negative feedback concerning FSH secretion occurs via the gonadal hormones inhibins, members of the transforming growth factor β super-family of molecules; Sertoli cells of the adult testes secrete both inhibins A and B, although the prominent FSH suppression occurs by inhibin B, which modulates activin-driven FSH stimulation [9]. Follistatins produced within the pituitary bind activins and further decrease their action. Beyond their negative pituitary feedback, inhibins function also throughout the reproductive hormonal axis and act locally as paracrine hormones within the testes [9].

15.1.2 Thyrotropic Axis

TRH-producing cells are located within the hypothalamic paraventricular nucleus, and TRH stimulates the synthesis and secretion of pituitary TSH, in a pulsatile and diurnal fashion, which in turn acts at the thyroid gland to stimulate all steps of biosynthesis and secretion of the thyroid hormones (THs), tetraiodothyronine (T4) and triiodothyronine (T3). T4 and T3 exert a negative feedback directly at the pituitary, by inhibiting TSH secretion from the thyrotropic cells, or at the hypothalamus, by inhibiting TRH secretion. Concerted action of TRH and thyroid hormones results in relatively stable circulating concentrations of TSH [10]. Thyroid activity increases during childhood and adolescence, due to its critical role in nervous system development, linear growth and metabolism; during this period, THs act on osteoblasts and osteoclasts by influencing bone formation and resorption [11], control protein, lipid, carbohydrate and vitamin metabolism, and oxygen consumption [12], and regulate testicular development by controlling Sertoli cells and Leydig cells differentiation and proliferation [13]. Only 0.03% of total serum T4 and 0.3% of total serum T3 are present in the free form in circulation, whereas the major aliquot is bound to thyroxine-binding globulin (TBG), thyroxine-binding prealbumin (TBPA) or transthyretin, and albumin. THs actions are mainly mediated by nuclear THs receptors (THR), which modify target genes expression. Free circulating T4 and T3 are taken up by target cells in peripheral tissues; T3 is the preferred ligand

of THR, whereas T4, whose serum concentration is 100-fold higher than that of T3, undergoes extra-thyroidal conversion to T3, an enzymatic reaction catalyzed by type I and II deiodinases (D1 and D2). T3 and T4 are inactivated by conversion to diiodothyronine (T2) and reverse T3, which has very low affinity for THR, by type III deiodinase (D3). Regulation of deiodinases, particularly D2, and THs transporters at the cell membrane, controls T3 availability [14].

15.1.3 Lactotropic Axis

TRH and additional hypothalamic polypeptides stimulate PRL secretion by the pituitary lactotropic cells, whereas formerly known prolactin-inhibiting factor (PIF) produced in the arcuate nucleus, now identified as dopamine, provides negative modulation of PRL. The axons of lactotropic cells project to the ME, where their products are secreted into the primary plexus and conveyed to the adenohypophysis via the portal system. The main mechanism of PRL regulation is via a direct tonic inhibition of the pituitary provided by dopamine, which inhibits the basal high-secretory tone of lactotropic cells. Hypothalamic dopaminergic neurons are regulated by short-loop feedback from PRL itself, whereas exercise, stress, sleep, pregnancy, and stimulation of the nipple, all increase PRL secretion. PRL, in conjunction with estrogen and progesterone, accounts for milk secretion from the female breast. Pituitary lactotropic cells are controlled by an indirect negative feedback exerted by PRL, which stimulates dopamine release from the ME, therefore ultimately reducing PRL level itself [15].

15.1.4 Corticotropic Axis

Hypothalamic CRH-producing cells located within the paraventricular nucleus stimulate ACTH secretion by corticotropic cells of the pituitary, ultimately resulting ACTH-induced adrenal corticosteroid secretion, in response to stress; corticosteroids, in turn, exert a negative feedback control both directly at the pituitary, by reducing ACTH, and indirectly at the hypothalamus, by reducing CRH and vasopressin secretion, resulting in the modulation of both basal and stress-induced ACTH secretion. Corticosteroids' negative feedback is mediated by mineralocorticoid receptors (MRs) and/or glucocorticoid receptors (GRs), located in multiple sites within the brain and pituitary [16], and the underlying mechanisms vary according to receptor type and location within the brain–hypothalamus–pituitary unit. A very rapid non-genomic action has been demonstrated for GR action on CRH neurons, whereas slower non-genomic actions occur at the pituitary or different brain sites mediated by GR and/or MR. Corticosteroids also have genomic actions targeting CRH and vasopressin genes within the hypothalamus and proopiomelanocortin (POMC) gene within the pituitary, which are repressed by corticosteroids. Dynamics of rapid and delayed negative feedback actions of corticosteroids also differs; rapid inhibition requires rapidly rising corticosteroid

levels, whereas delayed inhibition depends on the intensity of the stimulus and the magnitude of the corticosteroid feedback signal, and on the neuroanatomical pathways responsible for activating the corticotropic axis. Some physiological stressors may partially circumvent hypothalamic CRH neurons feedback sites, whereas different psychological stressors may ease responses to subsequent stress [16].

15.1.5 Somatotropic Axis

GHRH-producing cells are located within the hypothalamic arcuate nucleus and positively modulate GH secretion by the pituitary somatotropic cells, whereas a negative stimulus is provided by a different hormone, formerly growth hormone-inhibiting hormone (GIH), and now identified as somatostatin, which is produced in the paraventricular nucleus. The axons of these cells projecting to the ME release regulatory hormones to the primary plexus and therefore reach the adenohypophysis via the portal system [17]. The secretion of GHRH is episodic, whereas that of somatostatin is more tonic. GH secretion is enhanced by different stimuli, which include hypoglycemia, exercise, sleep, and various types of stress; conversely, GH secretion is inhibited by glucose and cortisol [17]. GH stimulates the production of somatomedins, a group of polypeptide growth factors secreted by liver, cartilage, and other tissues, including insulin-like growth factor 1 (IGF-I), which mediates the majority of GH actions, and peaks at puberty by declining to low levels in the elderly, and insulin-like growth factor II (IGF-II), which exerts a key role in fetal growth, but is only expressed in the choroid plexus and meninges, at adulthood. GH has pleiotropic effects throughout the body; before epiphyseal fusion in children, GH drives linear growth and chondrogenesis, but it is also an anabolic hormone in children as well as in adults [18]. GH stimulates human muscle growth, mainly by reducing the rate of protein oxidation and increasing protein synthesis, and stimulates lipolysis and lipid oxidation and reduces free fatty acids uptake in fat tissue, by resulting in increased lean body mass and decreased fat body mass [19, 20]. Moreover, GH increases glucose production through gluconeogenesis and glicogenolysis in the liver and suppresses glucose uptake in the skeletal muscle and in the adipose tissue by inducing insulin resistance, which is however counteracted by IGF-I [21]. Pituitary somatotropic cells are controlled by a direct negative feedback exerted by IGF-I, which also indirectly inhibits GH secretion by stimulating somatostatin secretion from the hypothalamus [17].

15.2 The Hypothalamic–Pituitary Axis Function in Klinefelter's Syndrome

Klinefelter's syndrome (KS) is characterized by the presence of one or more extra X chromosomes, with the 47, XXY karyotype being the most prevalent; the syndrome is typically associated to hypogonadism and male infertility [22]. Unlike autosomal trisomies, which a paternal origin in just 10% of cases, the supernumerary X chromosome in KS arises from paternal non-disjunction in 50% of cases, of which the vast

majority is linked to meiosis I non-disjunction events, apparently independent from Y chromosome deletions and rearrangements [23]. A distinct KS phenotype only becomes evident after puberty and, due to the paucity of symptoms, only 10% of patients are diagnosed pre-pubertally [22]; tall stature, small testes, androgen deficiency, gynecomastia, decreased muscle mass, decreased bone density, reduced facial and body hair, infertility, and an increased risk of metabolic disorders, cardiovascular abnormalities, and neuropsychiatric conditions, are the major clinical characteristics, many of which can be attributed to the genetically determined primary gonadal defect and to subsequent hypogonadism and androgen deficiency [22, 24–27].

The hypothalamic–pituitary axis function in KS has been scantly investigated, mainly by studies published in the years 60s-90s, and some data suffer from controversies, due to various factors which severely dampen knowledge of hypothalamus–pituitary circuits in KS patients, including the rarity of the condition and the underestimated diagnostic rate which contribute to the reduced availability of cases to be studied, the fact that most evidence derive from KS patients actively seeking medical consultation, which probably display the most severe degree of the condition, and the lack of recent and large clinical trials. Therefore, some aspects of hypothalamic–pituitary axis function described in textbooks and scientific literature are obsolete unresolved questions, which would need to be resumed.

15.2.1 Gonadotropic Axis

During childhood, pituitary–gonadal function is relatively normal in KS patients, and after pubertal activation of the hypothalamic–pituitary–testicular axis, FSH, LH, inhibin B, INSL3, and testosterone levels increase apparently in a normal way in the early pubertal stages, with a minority of patients showing delayed puberty [22]. Nevertheless, from mid-puberty onwards, testosterone decreases by reaching a plateau at typically low-normal healthy adult men levels, INSL3 decreases to lower than normal levels, and inhibin B decreases to undetectable levels, suggesting a testicular impairment; subsequently, LH and FSH increase to overt hypergonadotropic levels. Conversely, on average, estrogens and sex hormone-binding globulin (SHBG) are higher than normal [22, 28]. Concerning AMH, levels within the reference range are reported in KS patients from the time of mini-puberty in infancy until the age of expected puberty, whereas the physiological decline occurring with advancing puberty appears to be delayed, compared to healthy boys; AMH thereafter declines to pathologically low levels, most likely due to hyalinization of seminiferous tubules in relation to puberty, rather than caused by disrupted regulatory mechanisms at the level of the pituitary–gonadal axis [30]. Although some residual Leydig cells' responsivity to exogenous GnRH has been reported [31–33], adult KS patients display a variable degree of testosterone deficiency, ranging from clinically overt hypogonadism to normal androgenic status, and the diagnosis is generally made during the evaluation of secretory azoospermia; therefore, testosterone replacement therapy is required to prevent symptoms and sequels of hypogonadism in these patients [22, 28, 29]. Although diagnosis of KS frequently occurs incidentally during azoospermia work-up, and the

majority of KS patients are azoospermic, a less severe impairment of spermatogenesis has been reported, with diagnosis of severe oligozoospermia or residual single-residual foci of spermatogenesis within the testis; it was demonstrated that in about 8% of KS patients spermatozoa could be found in the ejaculate, and a successful sperm recovery by TESE could be achieved in 37.5% of patients [34].

KS patients display significantly higher basal, pulsatile and total LH and FSH secretion when compared with healthy subjects, and the number of LH, but not FSH, pulses is significantly higher, although no difference has been reported in the regularity of pulsing and the shape of secretion pulses. Therefore, in KS the entire hypothalamic–pituitary–gonadal gonadostat has undergone functional changes resulting in an altered set point, due to primary testicular failure [35]. Attenuated testosterone feedback in KS is responsible for the greatly amplified pituitary responsiveness to the trophic action of GnRH and may be in part responsible for the high levels of FSH and LH seen in such patients [36]. Indeed, a dated study demonstrated that a 4-h infusion of GnRH produced exaggerated responses for both LH and FSH, which were decreased after 6 weeks of treatment with long-acting testosterone esters administered weekly [37]. Moreover, it was demonstrated that, in GnRH-stimulated KS patients, the ratio between biologically active and immunoreactive LH decreased significantly from basal values, whereas testosterone replacement therapy was able to increase this ratio, in basally secreted LH, suggesting a potential imbalance between biologically active and inactive LH, in KS patients [38]. Nevertheless, no further studies confirmed this evidence. Interestingly, inhibin B levels in KS patients were found to be even lower than those of men with idiopathic and Y deletion-associated oligo-azoospermia, whereas FSH had an inverse pattern, suggesting multiple mechanisms involved in the gonadotropic axis control [39]. Lastly, a controversial debate exists, in few small and dated studies, concerning the existence of a positive feedback of estrogens at pituitary level, in KS patients, with some evidence favoring [40, 41] and others disavowing [36] this hypothesis. A schematic overview of gonadotropic axis regulation in healthy men and KS patients is provided in Fig. 15.1.

15.2.2 Thyrotropic Axis

KS has been associated with thyroid abnormalities, the genesis of which has yet to be fully clarified. Adult KS patients have significantly lower serum T4 levels, which tend to cluster around the lower limits of the reference range, and T4/T3 ratio, compared to age-matched men, without displaying a compensatory physiological increase in TSH; these data suggest that inadequate hypothalamic-pituitary control of thyroid function and secondary thyroid insufficiency might be a common trait of the KS phenotype [42]. A recent, large study in KS patients and non-KS hypogonadal men evaluated the potential pathogenetic relationship between thyroid dysfunction and hypogonadism [43]. KS patients had significantly lower serum T4 levels, and superimposable T3/T4 ratio, TSH, and testosterone, compared to non-KS hypogonadal men, pointing to an impaired production of T4 by the thyroid gland, due to causes other than underlying hypogonadism [43]. Controversial small

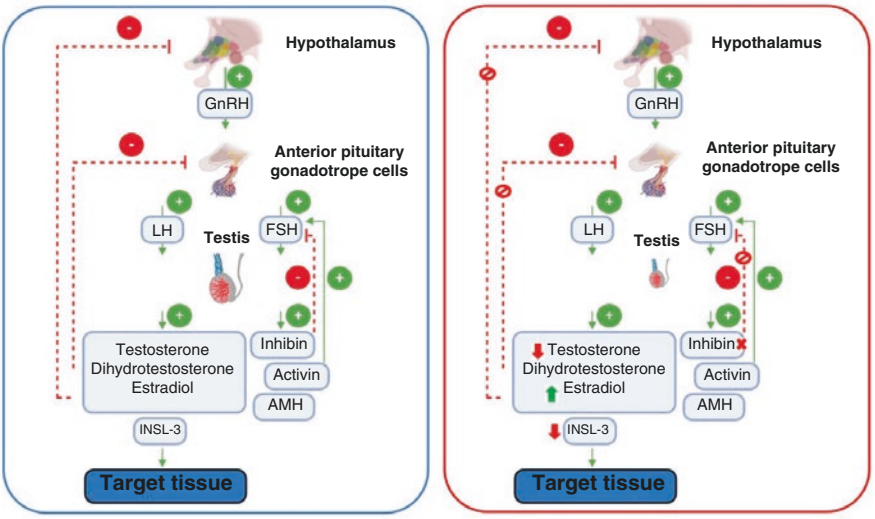

Fig. 15.1 Schematic overview of the gonadotropic axis regulation in healthy men (blue circled left panel) and patients with Klinefelter's syndrome (KS) (red circled right panel), figure created with BioRender.com. In physiological conditions, the major hormone controlling gonadotropin-releasing hormone (GnRH) secretion is testosterone, which inhibits gonadotropin secretion by exerting negative feedback at both the hypothalamic and pituitary levels; testosterone may act as such, or after conversion to dihydrotestosterone or estradiol. Inhibin also provides a negative regulation mechanism by selectively suppressing the release of follicle-stimulating hormone (FSH) from the pituitary gland. In KS patients, the entire hypothalamic–pituitary–gonadal axis has undergone functional changes resulting in an altered set point, due to primary testicular failure, and subsequent reduced testosterone levels, which prevents negative feedback mechanisms from suppressing gonadotropins secretion; as a result, luteinizing hormone (LH) and FSH increase to overt hypergonadotropic levels. Moreover, KS patients display undetectable inhibin levels, therefore further contributing to increase gonadotropins by the lack of inhibin negative feedback. Lastly, a debated hypothesis exists, concerning the presence of a positive feedback mechanism operated by estrogens, whose levels are generally high in KS patients, exerted at pituitary level

and dated studies reported either a lack of [44] or normal [45, 46] pituitary response to exogenous TRH administration, although the first consisted of a case report and the majority of studies highlighted a normal TRH response. One study evaluated thyroid function and pituitary response to TRH prior to and after testosterone treatment. The thyroid function tests, including serum T3, T4, TBG and resin T3 uptake (RT3U), radioactive iodine uptake (RAIU), response to TSH stimulation, and the TSH response to TRH, were normal both prior to and after testosterone treatment, which had no significant effect on any of these parameters [45]. These results were consistently demonstrated by different studies [46, 47], which also reported a significant rise in T3 at 3 h after TRH administration, confirming that thyrotropic axis is intact in KS patients [46]. Nevertheless, a case report on a clinically euthyroid KS patient failed to demonstrate TRH-induced TSH secretion, despite increased ^{131}I thyroid uptake following TSH administration, suggesting an extra thyroidal origin of THs dysfunction, resulting in impaired TSH secretion from the pituitary [44].

Lastly, few dated studies reported that hypothalamic-pituitary coordination of the circannual and circadian rhythmicity of TSH is apparently lost, in KS patients, since no circannual fluctuations of TSH were detected over a 3-year follow-up, in a quite large cohort [48], nor circadian fluctuations were detected over 24 h in a small series, although a pubertal pattern of hormone concentration with higher levels during the sleep period was found [49]. The thyrotropic and thyroid function has also been specifically evaluated in pubertal KS patients, which display significantly lower T3 serum levels, compared to healthy age-matched boys, whereas TSH and T4 are normal, albeit at the lower limits of the reference range. Moreover, an inadequate/prolonged response to pituitary stimulation by TRH was highlighted [50]. These evidences suggest a combined form of both central and peripheral hypothyroidism in KS boys during pubertal development [50]. In conclusion, scientific literature concerning the potential association between KS and thyroid dysfunction is not fully consistent; nevertheless, the most common finding in KS patients is the occurrence of reduced serum T4, along with normal serum TSH levels.

15.2.3 Lactotropic Axis

Clinical studies investigating the lactotropic axis in KS are few and controversial. Indeed, some studies reported significantly increased basal PRL levels and greater PRL secretion upon TRH administration in KS patients, compared to healthy men [45, 51, 52], whereas others reported normal basal PRL levels, despite an enhanced TRH-stimulated pituitary response [45], which was further increased by concomitant estrogen [53] or testosterone [54] administration; conversely, normal basal and TRH-stimulated PRL levels were reported by another study [55]. No direct association of KS with hyperprolactinemia has been noted, although sporadic PRL-secreting adenoma has been recorded in a case of KS [56]. In conclusion, no firm evidence exists to date on a potential lactotropic axis dysfunction in KS patients.

15.2.4 Corticotropic Axis

Extremely few and very dated studies evaluated the corticotropic axis in KS patients, and mostly present single case reports or very small series. It has been reported that, in KS patients, circadian cortisol rhythm is preserved, although at higher levels, compared to healthy men [57]. Moreover, in the attempt to determine whether the adrenal cortex might contribute to plasma testosterone levels in KS patients, short-term stimulation of the adrenal cortex by ACTH and suppression by dexamethasone was performed; the results showed that ACTH administration failed to increase testosterone levels; therefore, a prominent contribution of adrenal androgens to the low circulating levels of testosterone in KS is questionable [58]. In conclusion, the unique change highlighted by the scarce studies on corticotropic axis function in KS patients is a shift towards higher hormone levels in an otherwise preserved circadian cortisol rhythm.

15.2.5 Somatotropic Axis

KS patients are characterized by eunuchoid proportions, displaying longer legs and shorter trunk, compared to age-matched men, and exhibit final height above parental height-based predictions. Linear growth is a complex process resulting from the interaction of multiple factors, which include hormonal, metabolic, genetic, environmental, and socioeconomic aspects; a pivotal role in such process is exerted by the interplay between gonadotropic and somatotropic axes, and long-leggedness and impaired trunk growth are typically associated to hypogonadism. Although gonadotropic axis has been investigated in KS patients, mainly by dated studies, the somatotropic axis has been scantly regarded so far. A large study in KS patients demonstrated accelerated growth from early infancy throughout childhood and adolescence, not mirrored by increased circulating levels of IGF-I and insulin-like growth factor binding protein 3 (IGFBP-3), which were in the normal range; moreover, the abnormal growth pattern preceded hypogonadism, as hormonal evidence of impaired testicular function [59]. These evidences suggest that factors other than impaired somatotropic axis or hypogonadism might be the cause of KS patients' corporeal features, maybe an excessive expression of growth-related genes, due to the underlying X chromosome aberration [59]. Consistently, another report assessing the 12-h nocturnal GH profile in few KS patients found normal secretion and pulsatility of IGF-I and IGFBP-3, relative to height velocity and pubertal stage [60]. Lastly, GH levels did not significantly differ between KS patients and healthy men, after exogenous stimulation by GnRH and TRH [55, 61]. Nevertheless, some rare case reports described GH/IGF-1 resistance causing growth failure [62], GH deficiency [63–66] and acromegaly [67, 68]. In conclusion, no evidence of impaired somatotropic axis has been provided so far, in KS patients, and both higher stature compared to general population and eunuchoid proportions seem to be not associated to GH/IGF-1 system dysfunction.

15.3 Conclusions

Scant and somehow conflicting evidences, derived from outdated case reports and small case series, exist on the hypothalamus–pituitary axis function, in patients with KS. The less equivocal data concern gonadotropic and thyrotropic axes, whereas large inconsistency, mainly due to the absence of large studies, affects understanding of the lactotropic, corticotropic and somatotropic axes function and regulation. The first signs of an impaired gonadotropic axis alignment in KS patients occur from mid-puberty onwards, with testosterone levels decreasing to low-normal healthy adult levels, INSL3 decreasing to lower than normal, and inhibin B to undetectable levels; these changes, predominantly due to primary testicular damage, determine the failure of negative feedback mechanisms operating at the hypothalamus–pituitary unit, thereby leading to overt hypergonadotropism, with significantly higher basal, pulsatile and total LH and FSH secretion. Still controversial is the existence of a positive feedback exerted by estrogens at pituitary level, which might sustain gonadotropins excess. In adult KS patients, serum T4 levels usually cluster around the lower reference range limits,

without compensatory increase in TSH, suggesting an impaired production of T4 by the thyroid gland, which does not seem to be determined by hypogonadism, nor by deficient hypothalamus–pituitary control of thyroid function, as suggested by an apparent normal pituitary response to TRH administration. Nevertheless, one study in pubertal KS patient hypothesized a combined form of both central and peripheral hypothyroidism, due to an inadequate pituitary response to TRH administration. Great heterogeneity of data characterizes the pituitary lactotropic cells' responsivity to exogenous TRH, in KS patients, although the majority of studies report enhanced PRL secretion, compared to healthy men. Corticotropic axis has been almost completely disregarded, with a bunch of studies only focusing on the adrenal androgens production, and highlighting a preserved circadian cortisol rhythm. KS patients are typically characterized by tall stature and eunuchoid proportions, which are not likely determined by somatotropic axis abnormality, since accelerated growth occurring from early infancy throughout childhood and adolescence is not accompanied by increased circulating levels of IGF-I and IGFBP-3, nor by the impairment of gonadotropic axis, since the disproportional pattern of growth precedes biochemical evidence of hypogonadism. Moreover, normal secretion and pulsatility of IGF-I and IGFBP-3, and GH secretion in response to GnRH and TRH stimulation occur in KS patients. It is important to highlight that, despite investigation concerning the hypothalamus–pituitary unit in KS accounts obsolete studies, the most consistent evidence of an impairment is demonstrated only in regards of the gonadotropic axis, whereas no specific damage might be observed over other axes; this is largely consistent with real clinical practice and with routine or in-depth hormonal assessment. Probably, these evidences from the clinical practice also justify the lack of more recent studies on this topic.

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Klinefelter Syndrome: The Altered Bone

16

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16.1 Introduction and the Role of Hypogonadism

Osteoporosis is a silent condition, defined as a systemic skeletal disease characterized by low bone mass—generally measured as bone mineral density (BMD) with dual energy X-ray absorptiometry (DXA)—and bone micro-architectural impairment, leading to bone fragility.

Patients with Klinefelter syndrome (KS) have a high risk of developing osteopenia, osteoporosis and fractures [1, 2]. Decreased bone mineral density (BMD) is reported in about 25–48% of patients [3], whereas overt osteoporosis accounts for 6–15% of cases [4]. Low BMD in KS is due to both reduced bone formation and increased bone resorption [5–7]. The annual decrease in bone mass rate in KS has been calculated in 1.18 ± 0.53% at the lumbar level and 1.03 ± 0.43% at the femoral neck level [8].

Young KS subjects have normal BMD in childhood and at the beginning of pubertal development [2]. In fact, normal lumbar BMD and whole body bone mineral content (BMC) as evaluated by whole body DXA scan in KS boys and adolescents (4.3–18.6 years of age) has been reported, indicating that the risk of osteopenia/osteoporosis may not be present until after puberty [2]. However, studies evaluating the bone status and metabolism of children and adolescents with KS are very rare and not uniform. Later during puberty, they develop a progressive testicular failure, with subsequent primary hypogonadism. Such a deficiency in testosterone production and low plasma levels [3, 4] represent probably the most important risk factor

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for reduced bone mass and osteoporosis because it might compromise reaching the peak bone mass that normally occurs at the end of puberty [7]. Supporting this hypothesis, bone histology of KS hypogonadal subjects showed loss of cancellous tissue, profound depression of osteoblast activity, decreased osteoid seam width and slowing of the apposition rate [9], whereas these findings were not confirmed in KS subjects with normal testosterone levels [10]. Indeed, hypogonadism represents one of the most important causes of male osteoporosis and testosterone is known to represent a fundamental anabolic hormone.

However, the pathogenic role of low testosterone is still not well established [11] and conflicting data are reported [12]. Several studies showed that testosterone replacement therapy (TRT) in KS men with low testosterone levels and low BMD did not fully reverse bone density [13, 14], and this was more evident when TRT was started after puberty [4]. Conversely, other studies showed that starting TRT in young age (i.e. before reaching the peak bone mass) can lead to normal BMD [15, 16].

Indeed, no clear relation between testosterone serum levels and BMD has been found in patients with KS, and osteopenia/osteoporosis might also be present when testosterone concentrations are within the normal range [11, 12, 17]. Moreover, a similar prevalence of low BMD in KS men with both low and normal T levels exists [12], suggesting that bone loss in KS might be, at least in part, independent from the presence of hypogonadism. On this basis, other possible mechanisms contributing to osteoporosis in KS must be considered.

16.2 Low Insulin-Like Factor 3 (INSL3) Levels

Insulin-like factor 3 (INSL3) is a peptide hormone produced by pre- and post-natal Leydig cells [18–20]. It is mainly involved in the regulation of testicular descent during foetal development, by acting on gubernaculum via its specific receptor Relaxin Family Peptide 2 (RXFP2) [21]. In adult males, INSL3 is produced constitutively, in a differentiation-dependent manner, by the Leydig cells under the long-term Leydig cell differentiation effect of LH [18, 22, 23]. On this basis, INSL3 has been proposed as a specific marker of Leydig cell differentiation status [18, 20, 22].

The dynamic of circulating levels of INSL3 is very similar to that of testosterone. After birth, INSL3 increases at about 3 months of age under the increased levels of LH (minipuberty) [22]. Soon after, INSL3 declines to undetectable levels, remains low during infancy [24] and then progressively increases throughout puberty [23]. Finally, INSL3 levels in adulthood decline steadily throughout life, and at 75–80 years INSL3 concentration is reduced by about 40% with respect to levels found at 35–40 years [25].

Reduced plasma concentrations of INSL3 are seen in situations of undifferentiated or altered Leydig cell status or reduced Leydig cell number, such as in anorchid men, men with hypogonadism, infertility or obesity [18, 22, 25]. Although the exact role of post-natal INSL3 is not fully understood, the general hypothesis is that reduced INSL3 activity (caused by altered testicular function, *INSL3* or *RXFP2* gene mutations) could cause or contribute to some symptoms and signs of

hypogonadism, such as reduced BMD. In fact, INSL3 has been shown to exert an anabolic effect on bone during adulthood, by acting directly on osteoblasts and osteocytes [26–28], through RXFP2 activation. Interestingly, *Rxfp2*$^{-/-}$ mice showed decreased bone volume and altered trabecular organization at both lumbar and femoral sites [26]. In addition to its anabolic effect on osteoblasts, INSL3 also exerts positive effect on osteoclastogenesis, via increasing macrophage colony-stimulating factor (M-CSF) [27]. Therefore, the net effect of INSL3 on the bone is to maintain the osteoblast/osteoclast balance.

Few studies, to date, examined INSL3 levels in subjects with KS. Only one study examined INSL3 levels during puberty in boys with KS, showing a normal increase in serum INSL3 at initial stages of puberty and then a levelling off [29]. In adult KS subjects with reduced testosterone levels, also a very low level of INSL3 was reported [18], even if discordant results on its correlation with low BMD were obtained [30]. A recent study [28] found a strong inverse association between circulating levels of INSL3 and sclerostin (SOST), an osteocyte-specific protein that favours bone resorption, in a cohort of KS subjects and age-matched healthy men. Since the role of SOST in the pathogenesis of osteoporosis is well established [31], these data suggest that low INSL3 levels in KS subjects may alter bone quality by dysregulating SOST pathway [28].

Taken together, these findings, although preliminary, would suggest that the low INSL3 levels observed from mid-puberty onwards in KS could have a role in the reduced bone density and osteoporosis in these subjects.

16.3 Low Vitamin D Levels

Vitamin D might be another possible modulator of bone metabolism in KS. It is involved as a key regulatory factor in calcium homeostasis and bone metabolism in both men and women [32]. Biologically active form of vitamin D, 1,25-dihydroxyvitamin D (calcitriol), comes from two sequential hydroxylation steps, catalysed by 25-hydroxylase and 1α-hydroxylase. When vitamin D levels are inadequate, calcium absorption is lower than optimal, which determines a compensatory increase in PTH levels (secondary hyperparathyroidism), and a subsequent stimulation of bone reabsorption and accelerated bone loss [12]. Interestingly, the male reproductive tract expresses most of the enzymes involved in vitamin D metabolism. In particular, the testis has been shown to have the highest expression of CYP2R1 enzyme, a member of cytochrome P450 family [33] with a pivotal role in vitamin D activation through its 25-hydroxylase activity [34]. A pathophysiological link between testicular damage, low 25-hydroxyvitamin D levels and reduced bone mass has been shown, supporting the physiological importance of CYP2R1 expression in the testis [35]. Impairment of testicular function leads, therefore to low levels of 25-hydroxyvitamin D and consequently to an increased risk of osteopenia and osteoporosis [35]. Few reports determined 25-hydroxyvitamin D levels in KS [12, 27] demonstrating that KS subjects have 25-hydroxyvitamin D levels lower than healthy controls.

Low 25-hydroxyvitamin D levels in KS subjects could be interpreted as a consequence of the severe testicular hypotrophy and Leydig cell impairment, which are characteristic signs of these subjects. Furthermore, lumbar and femoral BMD in KS were positively associated with 25-hydroxyvitamin D in a recent study [12], and patients with 25-hydroxyvitamin D deficiency had lumbar and femoral BMD significantly reduced with respect to KS subject with 25-hydroxyvitamin D ≥ 50 nmol/L [12]. It has also been demonstrated that correcting a vitamin D deficiency is also fundamental to maintain BMD in KS men, bot hypogonadal and eugonadal [12].

16.4 Unfavourable Fat/Muscle Ratio

Another possible mechanism involved in the development of bone loss in KS might be related to the unfavourable fat/muscle ratio caused by increased fat mass and reduced muscle mass [1, 36]. However, it is not fully understood whether such an altered ratio is caused exclusively by the low testosterone levels or by other mechanisms related to the genetic defect. In fact, studies suggested that the unfavourable fat/muscle ratio is already present in young adolescents, whereas bone mass defects appear in from late puberty onwards [2]. Indeed, TRT is known to increase skeletal mass and strength and these in turn are fundamental to maintain also bone homeostasis.

16.5 Androgen Receptor CAG Polymorphism and Non-random X Inactivation

Testosterone regulates bone metabolism both directly (after transformation to dihydrotestosterone—DHT—by 5α-reductase), through the androgen receptor (AR) action on osteoblasts and osteocytes, and indirectly, through aromatization into oestrogens. Therefore, the AR function and sensitivity might modulate the effects of testosterone on the bone [37]. The AR gene is located on the X chromosome, and a non-random X inactivation in men with more than one X chromosome has been observed [38]. The first exon of the AR gene encodes for the transactivation domain of the AR protein. It contains the highly polymorphic CAG repeat, the length of which is inversely correlated with androgen sensitivity [39]. Therefore, in KS the CAG repeat length depends on the inactivation rate of the two X-chromosomes [38]. As a consequence, different clinical outcomes and the response to testosterone therapy (BMD, gynecomastia, testes and prostate volume, haemoglobin concentration) have been associated with AR CAG length in KS [38]. Nevertheless, conflicting data on the relation between CAG polymorphism of the AR and bone metabolism in KS have been published. Whereas a study found it to be negatively and independently associated with BMD [38], another study did not confirm that finding, nor that KS patients had a non-random X inactivation [37], and there were conflicting results regarding the relation between BMD and CAG even in normal men [40]. Furthermore, also the role of CAG length in regulating androgen sensitivity has

been questioned [40], since an inverse correlation between CAG length and androgen sensitivity was generally assumed [39], but it was showed that the CAG polymorphism is not related to AR activity in vitro [41].

A certain degree of androgen resistance has previously been reported in KS [3, 42], with a decreased activity of bone 5α-reductase [43] and a lower peripheral AR expression on lymphocytes [44], testis [45] and smooth muscle cells [46]. However, the AR expression in bone has never been studied in KS. A potential decrease in AR expression in bone could explain, at least in part, the frequent ineffectiveness of TRT in improving BMD in KS. Besides, as differences in androgen signalling and AR expression in vertebral (trabecular) and femoral (cortical) bone are well known [47], a reduced AR expression in bone generally results in a further decrease in BMD of trabecular rather than cortical bone. Indeed, the AR pathway is particularly effective in the trabecular bone, where androgens maintain or increase trabecular number and suppress trabecular reabsorption [48].

Evidence on AR CAG polymorphism and non-random X inactivation is, therefore, still not conclusive, and further studies are needed to clarify possible AR functional abnormalities in KS, AR expression in the bone, and their relationship with other potential determinants of reduced bone mass, such as the aforementioned INSL3 and vitamin D levels [49].

16.6 Low Oestrogen Levels

Oestradiol is known to be a central regulator of bone turnover [50], and many observational studies in men have documented that oestrogen levels are more closely correlated with BMD and bone turnover markers than serum testosterone levels are [51].

Even if oestradiol levels are generally normal or high in KS subjects, low oestrogen levels have been related to decreased bone mass in these patients in some studies [52, 53] and oestradiol levels are inversely related to the rate of bone loss [8]. However, these data have not been replicated in further studies and conclusions on this possible pathogenic mechanism cannot be made.

16.7 High Follicle Stimulating Hormone (FSH)

Follicle-stimulating hormone (FSH) function has long been thought to be exerted only in gonadal tissues, mainly limited to Sertoli cells in testes and granulosa cells in ovaries. More than a decade ago, however, it has been suggested that elevated FSH levels with concomitant declining sex steroids concentrations were responsible for the development of osteoporosis [54]. More recently, FSH receptors (FSHRs) have been shown to be expressed in extragonadal tissues, including endothelium, monocytes, malignant tissues, bone and fat [55].

Based on studies in post-menopausal women, there is some evidence that FSH may have profound effect on bone, by influencing pro-inflammatory and

pro-osteoclastogenic cytokine expression, such as receptor activator of NF-κB (RANK) [56]. More recently, a possible link between FSH and RANKL in men with KS has been proposed [57]. Normally, RANKL is a transmembrane protein of osteoblasts that binds and activates RANK located on osteoclasts, therefore inducing osteoclastogenesis [58]. Moreover, it has been shown that even bone marrow adipocytes produce RANKL and release it into bloodstream, under the influence of PTH [59]. In men with KS an inverse association between RANKL and FSH values was found, and the FSH/RANKL-ratio was significantly higher in KS subjects than in non-KS infertile men, even if KS patients showed immensely higher levels of serum RANKL [57]. Indeed, men with KS reach very high FSH levels, but also have a high degree of adiposity and prediabetes. This may explain why KS patients had higher RANKL despite of high FSH levels, taken into account FSH stimulation on RANKL shedding by adipocytes [57]. Another study has shown that KS adolescents have impaired bone mineral status with low osteocalcin and high PTH [56], which may indicate that the higher RANKL also could be due to higher PTH value.

Taken together, these data may suggest that many factors are implied in determining soluble RANKL levels and that FSH might be a potential bone regulator in KS.

16.8 Bone Quality and Risk of Fracture

The refinement of bone imaging methods, such as high resolution peripheral quantitative computed tomography (HRpQCT) has allowed the evaluation of cortical and trabecular micro-architecture in very high details, and the determination of three-dimensional volumetric BMD (vBMD). Also, finite element analysis (FEA), a biomechanical computation model, can be applied to HRpQCT images to obtain estimates of bone strength, thus allowing more insights into bone micro-architecture without the need for an invasive bone biopsy [60]. To date, two studies have assessed vBMD, micro-architecture and estimated bone strength in KS [60, 61], with divergent findings on BMD, but with concordance on bone strength alteration. This may suggest, for KS patients, a higher risk of developing fragility fractures independently from BMD values. Although the exact fracture rates are unknown, some epidemiologic studies have shown a correlation between fractures and increased morbidity and mortality in KS [1, 62, 63]. Further targeted studies are needed to evaluate fracture rates and fracture risk in KS patients. Moreover, no studies have been performed in osteoporotic KS subjects with fractures as primary outcome on treatment intervention.

16.9 Conclusion and Perspectives

Decreased bone mass in men with KS is of course multifactorial. Reduced testosterone levels play undoubtedly an important role, but other determinants, such as low INSL3, 25-hydroxyvitamin D and oestrogen levels, unfavourable fat/muscle ratio,

high FSH levels, a genetically determined reduced androgen action on bone by a non-random X chromosome inactivation and different CAG length polymorphism of the AR gene might cooperate and modulate the effect of testosterone. Importantly, the combined effect of all these factors acts early during KS life, mainly at the middle-end of puberty and during young adulthood. This might impact reaching the peal bone mass, therefore representing the pathogenic mechanisms leading to the precocious decrease in bone mass in KS patients. Therefore, it is essential not to underestimate bone health KS patients, and proactively prevent bone loss as early as possible, assessing vitamin D levels from childhood, and providing adequate supplementation in case of deficiency. Furthermore, from puberty onwards, it is recommended to monitor patients with KS with DXA analysis and serum bone markers, according to guidelines for bone health in andrological patients [64], keeping in mind that KS men are at risk of low BMD values and fractures regardless, at least in part, of testosterone values.

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Obesity: The Rule or Not

17

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17.1 Introduction

Obesity is one of the leading public health issues worldwide since associated with several diseases, including coronary heart disease, cerebral vasculopathy, type 2 diabetes mellitus, arterial hypertension, and dyslipidemia, which contribute to a reduction of both life quality and expectancy [1]. A rapid increase in the global rates

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of overweight and obesity in both men and women of all ages has been reported in the last 40 years, which has further increased the burden of this disease [2–4]. Nevertheless, achieving a weight loss of at least 5–10% is associated with significant clinical benefits on most of the obesity-related comorbidities and consequences, including death [5–8]. Accordingly, current guidelines recommend the definition of a clinical care pathway on an individual basis to reach this target. Lifestyle measures, including diet and physical activity, should be pursued in all subjects, while medications and bariatric surgery should be proposed to those patients meeting specific criteria [4, 9–11].

The definition of obesity and the exclusion of those secondary forms are keys. According to the World Health Organization, the ratio between the body weight (kilograms) and the height (meters square) defines body mass index (BMI). An adult subject can be defined as being overweight if BMI 25–29.9 kg/m^2 and as obese if BMI $\geq$ 30 kg/m^2. A child or a teen can be defined as being overweight if BMI 85th–94.9th and as obese if BMI $\geq$ 95th percentile for the same age and sex. It is commonly used as a measure of adiposity; however, it cannot discriminate neither between fat mass and fat free mass, nor between subcutaneous or adipose tissue [3]. Following the diagnosis of obesity, a clinical assessment for underlying diseases, such as Cushing syndrome, growth hormone deficiency, hypogonadism, hypothalamic obesity, hypothyroidism, monogenetic or syndromic obesity, should be performed; although rare, these disorders warrant a specific approach. Also, the contribution of medication and psychiatric disorders should be evaluated [12].

Among the phenotype features of subjects with Klinefelter syndrome, overweight and obesity are commonly included. The underlying pathogenic mechanism has not been fully described; however, it is likely that both the supernumerary X chromosome and testosterone deficiency play a role by inducing a vicious cycle of skewed metabolism, insulin insensitivity, and deposition of abdominal fat with a further negative effect on androgen status [13, 14]. The hypothesis that the genetic composition in Klinefelter syndrome could be directly affecting the risk of obesity, independently of hypogonadism, is supported by the finding of increased body fat and decreased lean body mass already in affected boys among whom hypogonadism is relatively less pronounced compared with healthy boys, and by the finding that deposition of fat is still increased in these subjects compared with controls after sufficiently adequate testosterone replacement therapy [15, 16]. Later in life, low socioeconomic status could contribute to a higher risk of obesity, as men with Klinefelter syndrome compared with controls receive less education, have a higher risk of living alone and have a lower annual income [16]. Also, neuropsychiatric aspects are common in these patients, possibly being associated with eating disorders [17]. Worth of note, among the genetic forms of obesity, Klinefelter syndrome is not included [12]. Then, we raise the question whether the relationship between the two disorders is supported by the available evidence. To avoid any bias related to testosterone replacement therapy, we aimed to address the following two questions: (1) Is obesity a common feature of Klinefelter syndrome at presentation? (2) Are subjects with Klinefelter syndrome at presentation more obese than the general male population?

17.2 Available Data on the Prevalence of Obesity

This was a narrative review. We performed separate searches in PubMed and Google Scholar on November 2019 to find evidence on the prevalence of obesity in subjects with Klinefelter syndrome. Obesity was defined as BMI ≥ 95th percentile for the same age and sex in children/teens and BMI ≥ 30 kg/m^2 in adults. Papers presenting data dichotomously were included (e.g., the number of obese subjects on the overall number of patients with Klinefelter syndrome). Studies reporting data on BMI as continuous variable (e.g., mean and standard deviation or median and range) were excluded. No language restriction was adopted. Two investigators (MC, VAG) independently searched papers, screened titles, and abstracts of the retrieved articles, reviewed the full texts, and selected articles for their inclusion.

To date, excluding case reports, only limited data have been reported in the literature on this topic. In 1966, Becker et al. reviewed the medical records of 50 patients with Klinefelter syndrome seen at the Mayo Clinic from 1956 to 1962. The average age was 38 years (range 17–66) and 50% were described as "significantly obese." However, the criterion for the diagnosis of this condition is unclear [18].

A first study was performed in Argentina. Medical records of patients with a confirmed diagnosis of Klinefelter syndrome and referred to the endocrinologist in five different centers in Buenos Aires from 1982 to 2008 were retrospectively reviewed. Data at presentation from 98 subjects were available. Among those 44 subjects aged less than 18 years at referral (range 0.7–17 years), a BMI above the 95th percentile for the same age and sex was found in one prepubertal and seven pubertal boys (18%). Among those 54 subjects aged at least 18 years at referral (range 18–60), a BMI ≥ 30 kg/m^2 was found in six patients (11%); moreover, 19 patients were overweight (35%). Therefore, the overall prevalence of obesity at presentation in this study was 14% [19]. A second study was performed in South Korea. Medical records of patients with a confirmed diagnosis of Klinefelter syndrome and aged ≥18 years in 11 university hospitals from 1994 to 2014 were retrospectively reviewed. Data at presentation from 376 subjects aged from 18 to 53 years (median 32) were available. Among these patients, a BMI ≥ 25 kg/m^2 was found in 160 patients (43%) and reduced total testosterone was found to be an independent risk factor for obesity. In 2000, a recommendation for lower BMI cut-off values in Asian populations was made by the World Health Organization, and a BMI ≥ 25 kg/m^2 was indicated to diagnose obesity. This recommendation was not confirmed in following statements [20]. Therefore, the overall prevalence of obesity at presentation in this study should be lower than the reported one of 43% [21].

Another relevant aspect to be assessed is the following. Is obesity more common in patients with Klinefelter syndrome at presentation compared to matched controls? No comparison with age-matched healthy controls was performed in any of the two studies above. Regarding the former, the prevalence of obesity in the general adult Argentinian male population in 2000 was about 20%, then it can be argued that Klinefelter syndrome is a risk factor for obesity [3]. Concerning the latter, the prevalence of obesity in the general adult Korean male population in the same time period ranged from 25 to 38%, then the authors concluded the prevalence of obesity

among patients with Klinefelter syndrome to be higher than the general population [21].

The heterogeneity of these findings does not allow a definitive conclusion to be drawn. While the reliability of the results of the study performed in South Korea is supported by the significant number of included patients, the same does not hold true for the study conducted in Argentina. Also, the sample size of the latter study supports the internal validity of the study itself and possibly its external validity to all subjects with Klinefelter syndrome in South Korea, but no inference should be drawn on other countries. In conclusion, still no adequate answer to the question if patients with Klinefelter syndrome are obese at presentation or more obese than general population can be obtained.

17.3 Unpublished Data on the Prevalence of Obesity in Patients from Denmark, Italy and the Unites States of America

We reviewed our institutional databases for the prevalence of obesity at presentation in adults with Klinefelter syndrome. All procedures were in accordance with the local ethical standards and with the Declaration of Helsinki; all participants included in this study gave their informed consent. From the Department of Endocrinology and Internal medicine, Aarhus University Hospital (Denmark), data from two previously published cohorts of men with Klinefelter syndrome recruited from 2002 to 2004 and from 2009 to 2012 were included [22, 23]. The median age of the first group was 35 (range 19–66), while the mean (standard deviation) age of the second group was 37 ± 10 years. Out of a total of 49 men with Klinefelter syndrome not on testosterone replacement therapy, nine (18%) presented with a BMI ≥ 30 kg/m^2. Medical records from 2010 to 2018 in the Outpatients Clinic of Endocrinology and Metabolic Disease, Conversano Hospital (Italy), were analyzed. Twenty-five subjects were found with a median age of 29 years (range 22–36) and one (4%) had a BMI ≥ 30 kg/m^2. Also, medical records of pediatric subjects participating in a previously published randomized controlled trial on oxandrolone versus placebo performed at Thomas Jefferson University, Philadelphia (Pennsylvania, the Unites States of America) from 2007 to 2011 were obtained after contacting the corresponding author [24]. In this study, 93 children with no treatment with exogenous androgens in the previous year were included. The median age was 7 years (range 4–12) and 12 (13%) had a BMI ≥ 95th percentile for the same age and sex.

We were not able to compare these findings with matched controls. However, in the same period the prevalence of obesity in adults in Denmark and Italy was about 20% and the prevalence of obesity in prepubertal children in the Unites Stated of America was about 18% [3, 25]. The data do not support a higher prevalence of obesity in subjects with Klinefelter syndrome or its contribution as cause of secondary obesity.

17.4 Should Patients with Klinefelter's Syndrome and Overweight/Obesity Be Treated with Specific Lifestyle Measures?

To date there is a lack of studies specifically conducted in patients with Klinefelter syndrome and overweight or obesity. However, it is common experience that these patients often develop metabolic syndrome and type 2 diabetes mellitus [16, 26]. Therefore, evidence from studies conducted in patients with these disorders can be reasonably adopted, although specific contributions stemming from the genetic disposition in Klinefelter syndrome cannot be evaluated. Lifestyle interventions for metabolic syndrome and type 2 diabetes mellitus should be designed to induce weight loss and mitigate the underlying modifiable risk factors (e.g., physical inactivity). Indeed, a weight loss of 5–10% has been shown to improve blood pressure, lipid profile, liver enzymes, obstructive sleep apnea syndrome severity as well as to slow the progression of type 2 diabetes mellitus, improve glycemic control, reduce medication use, and induce disease remission [27–31].

Current guidelines recommend a 500–750 kcal/day energy deficit to achieve a body weight loss of 0.5–1 kg/week [29]. This deficit is attained by combining diet and physical activity. It is worth noting that in most adults with type 2 diabetes mellitus, 150 min or more of moderate-to-vigorous intensity aerobic activity per week is recommended, corresponding to 800–1200 kcal/week (about 150 kcal/day). Therefore, since physical activity allows only a modest increase in energy expenditure, calorie restriction by the means of a diet is mandatory. Concerning macronutrients composition, different types of foods can be restricted and high-/low-carbohydrate/fat/protein diets obtained. A never-ending debate is there on which type of diet is associated with better outcomes. A practical consideration could be that all diets are effective if they create the necessary energy deficit. However, according to recent meta-analyses, the Mediterranean diet seems to be the most effective dietary approach for glycemic control and management of dyslipidemia [32–35]. Individualized meal planning focusing on personal preferences, needs, and goals is key.

Common dietary advices are represented by the control of dietary fats and carbohydrates, the increased intake of fiber, alcohol moderation and sodium reduction. Fat intake should be limited to 35% of total calories. Eating foods rich in long-chain $n-3$ fatty acids, such as fatty fish, nuts and seeds is recommended; trans-fat intake must be avoided; saturated fats should be limited to 10% of total calories; cholesterol intake should be below 300 mg/day. Concerning carbohydrate, foods with a low glycemic index and a high fibrate content should be preferred, including vegetables, fruits, legumes, whole grains, as well as dairy products. The consumption of sugar-sweetened beverages (including fruit juices) and foods with added sugar should be minimized. Indeed, dietary fiber (particularly of the soluble type), which can be found in legumes, fruits, vegetables, and wholegrain cereals (e.g., oats and barley), has been associated with several beneficial effects (e.g., satiety) [36].

Alcohol consumption should be limited to one drink a day for women and two for men. Finally, a variable sodium reduction is recommended depending on the underlying disorders [27–31].

17.5 Limitation of the Present Review

In present review, we adopted the BMI as the criterion to diagnose obesity in subjects with Klinefelter syndrome. However, overweight and obesity are continuous variables and BMI may have a limited applicability in this specific population. Indeed, men with Klinefelter syndrome commonly present with above average height and reduced lean body mass due to reduced anabolism as a result of hypogonadism [16, 22]. For any given BMI men with Klinefelter syndrome will present with higher truncal fat mass compared with controls [22, 23]. Also in a recent randomized, double-blind, placebo-controlled, BMI-matched, cross-over study, 13 males with Klinefelter syndrome treated with testosterone for 6 months showed no changes in BMI compared with placebo treatment but considerable reductions was seen in abdominal fat evaluated by both computed tomography and dual-energy X-ray absorptiometry [37]. Finally, to our knowledge there are no available studies directly linking for instance quartiles of BMI and incidence of specific diseases in men with Klinefelter syndrome. This complicates applying BMI as a measure of obesity or a marker of morbidity risk in Klinefelter syndrome. As an example, boys with Klinefelter syndrome have been found to present with increased abdominal fat but BMI within the normal reference range [15].

Our criterion followed the World Health Organization indications. Also, it is common experience that in different settings, a cut-off to be used in clinical practice is reported in the guidelines on other continuous variables (e.g., hypogonadism) and it follows that obesity too can be approached as a dichotomous variable rather than a continuous one.

17.6 Conclusions

Recently, there has been a surge in the worldwide prevalence of overweight and obesity causing, in turn, a rise in metabolic disorders (dyslipidemia, metabolic syndrome, type 2 diabetes mellitus, etc.) and cardiovascular disease. Although uncommon, excluding secondary forms of obesity is mandatory since specific pathogenetic mechanisms can be tackled in these patients. Concerning the Klinefelter syndrome, there has been a long debate on whether obesity represents the rule or not in affected subjects and consequently if this genetic disorder should be included among the secondary causes of obesity. Limited and inconclusive evidence on the prevalence of obesity in men with Klinefelter syndrome has been reported in the literature so far. In addition, unpublished data from some western centers seem to point out that obesity defined as BMI $\geq$ 95th percentile for the same age and sex in children/teens and BMI $\geq$ 30 kg/m^2 in adults is not more frequent in Klinefelter subjects when

compared to the general population. However, in the light of the known limitation of adopting BMI as criterion to diagnose obesity in these subjects, further epidemiological studies are needed to try to fulfil the clinical issue about the prevalence of obesity in naïve subjects with Klinefelter syndrome. A better definition of obesity in Klinefelter syndrome is perhaps needed, and future epidemiological studies should seek to assess various measures of fat deposition and relevant clinical outcomes such as metabolic and cardiovascular disorders. The goal being to provide clinicians with a superior tool for risk assessment and lifestyle guidance in the individual patient.

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Lipids and Glucose Metabolism

18

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18.1 Introduction

Subjects with Klinefelter syndrome (KS) develop several metabolic abnormalities more frequently than normal males. Indeed, a higher prevalence of obesity [1], type 2 diabetes (T2D) [2, 3], dyslipidemia [4], and metabolic syndrome [5, 6] is observed in KS subjects compared to XY males. In a study obtained from a Danish registry, the risk of developing T2D was almost four times higher in men with KS than in age-matched controls [2]. Interestingly, type 1 diabetes (as well as other autoimmune diseases) was also found to be more prevalent in subjects with KS compared to controls [7]. In fact, endocrine humoral immunoreactivity seems to be not infrequent in KS patients and more often directed against autoantigens related to type 1 diabetes (i.e., GAD65 and IA2), with a higher positivity of the related auto-antibodies compared to controls (8% vs. 1%) [8].

The higher prevalence of metabolic disorders, together with several other complications (e.g., cerebrovascular diseases, thrombophilia, and osteoporosis) and poorer socioeconomic status, is likely responsible for the 40% higher mortality of KS subjects [9–12].

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18.2 Hormonal Factors

The factors causing the increased prevalence of metabolic disorders in KS have not been completely clarified. Multiple mechanisms, including increased truncal adiposity and socioeconomic disadvantages, may be involved in the development of metabolic abnormalities, which however are only in part the direct consequence of hypogonadism per se. The negative correlation between low testosterone (T) levels and obesity is well established [13–16]. In the general population, it is known that reduced T levels cause an unfavorable change in body composition, primarily through increased truncal fat and decreased muscle mass [17, 18], and a reduced muscle strength associated to an unfavorable muscle/fat ratio is also evident in KS subjects [4, 19]. Indeed, for any given body mass index (BMI), men with KS have a higher proportion of body fat than 46-XY peers, on average ~8% more fat on the trunk compared with controls [17].

Androgens affect several biological functions related to adipose tissue remodeling and energy storage (i.e., lipid uptake, lipogenesis/lipolysis, adipogenesis, and mitochondrial function). In the visceral adipose tissue of sedentary obese men, plasma levels of bioavailable T appear to be inversely correlated to the activity of lipoprotein lipase (LPL) [20], an important enzyme involved in lipid uptake and whose abnormal activity is linked with obesity [21]. Conversely, long-term T replacement therapy (TRT) in hypogonadal men results in a decrease in both LPL activity and triglyceride uptake in visceral abdominal adipose tissue with a consequent shift of lipids to the subcutaneous fat [22], indicating that reduced T levels may enhance triglyceride storage in visceral fat. In a genetic mouse model lacking the androgen receptor (AR), lipolysis rates are reduced and, consequently, visceral adiposity is observed [23–25]. Moreover, mice with a selective fat tissue AR knockdown (fARKO) develop metabolic dysregulation with early insulin resistance/hyperinsulinemia on a normal chow diet [26]. When these mice received a high-fat diet, insulin deficiency, hyperglycemia, and visceral obesity with altered expression of metabolic targets were apparent [26]. Accordingly, experimental studies suggest that T may regulate the commitment of adipose tissue stem cells favoring their differentiation toward a myogenic phenotype [27].

Hypogonadal men typically exhibit an increased visceral fat mass [37], and evidence from epidemiological studies suggest that visceral fat accumulation is associated with increased metabolic risk [38]. Moreover, reduced T levels are associated with a higher risk of metabolic syndrome [28]. For example, patients undergoing androgen deprivation therapy after surgery for prostate cancer develop diabetes 1.4 times more frequently and display higher grade insulin resistance than controls (i.e., both eugonadal subjects and subjects with prostate cancer treated by prostatectomy alone) [29, 30]. However, the negative association of T with T2D incidence in the absence of any association with LH and free T suggests that low T in men who develop T2D may be a marker of the disease rather than a causal risk factor [31].

Conflicting results exist concerning the association between circulating sex hormone-binding globulin (SHBG) and T levels and T2D in men [31–34].

Intriguingly, high SHBG levels have been reported to be associated with lower risk of T2D [34, 35]. On the other hand, low estrogen (E) levels that characterizes hypogonadism in men [36] may promote the development of T2D by triggering metabolic tissues, particularly the adipose tissue [36]. However, KS subjects display higher levels of both SHBG and E compared to their peers [37], raising the question whether the higher incidence of metabolic abnormalities derive exclusively from hypogonadism.

18.3 Genetic Factors

Studies using individuals with idiopathic hypogonadotropic hypogonadism (IHH) as a comparison group to those with KS found a lower incidence of T2D in the IHH cohort, suggesting the contribution of genetic factors [3]. Most genes on the redundant X chromosome(s) are subject to X inactivation, but there is a pseudo-autosomal region containing multiple genes that escape inactivation. Some of the phenotypic features observed in men with KS are thought to be related to genes in this pseudo-autosomal region. The short stature homeobox-containing gene (SHOX), for example, seems to be responsible for the increased stature often seen in KS, in a dose-dependent manner [38]. However, the function of many other genes in the pseudo-autosomal region remain unclear. The association of more severe karyotypes (48 or 49 chromosomes) with even higher prevalence of T2D (up to 57%) suggests a role of the supernumerary X chromosomes in the onset of hyperglycemia [3, 39]. However, KS is characterized by DNA methylation changes across the entire genome [40], and this may further contribute to the association with T2D. Indeed, a recent study found that the majority of the 363 significantly deregulated genes in men with KS in fact were not located on the X chromosome [41]. Thus, it is possible that the genetic basis of the association between KS and T2D may involve subtle changes in the epigenome and transcriptome rather than alterations of single genes on the supernumerary X chromosome.

Decreased muscle mass and increased fat mass in KS may be consequences of hypogonadism, as discussed. However, some of the changes in body composition start before puberty, suggesting also a role of non-hormonal factors [4]. Even if hypogonadism is usually not apparent until early adulthood, visceral obesity and metabolic syndrome may be seen early in children and boys with KS. In a study on 89 prepubertal KS boys, 37% and 24% of them were found to have elevated low-density lipoprotein cholesterol and insulin resistance, respectively, and 7% met the criteria of the metabolic syndrome [5]. Of note, this association might be explained by a reduced level of physical activity noticeable in these subjects already at this young age [5].

Whether the association of KS with T2D and the metabolic syndrome could be explained by androgen deficiency and/or genetic abnormalities is unclear (Fig. 18.1). Data obtained from prospective studies indicate that the phenotype of KS subjects may vary considerably, presenting with evident and classic characteristics (i.e., tall

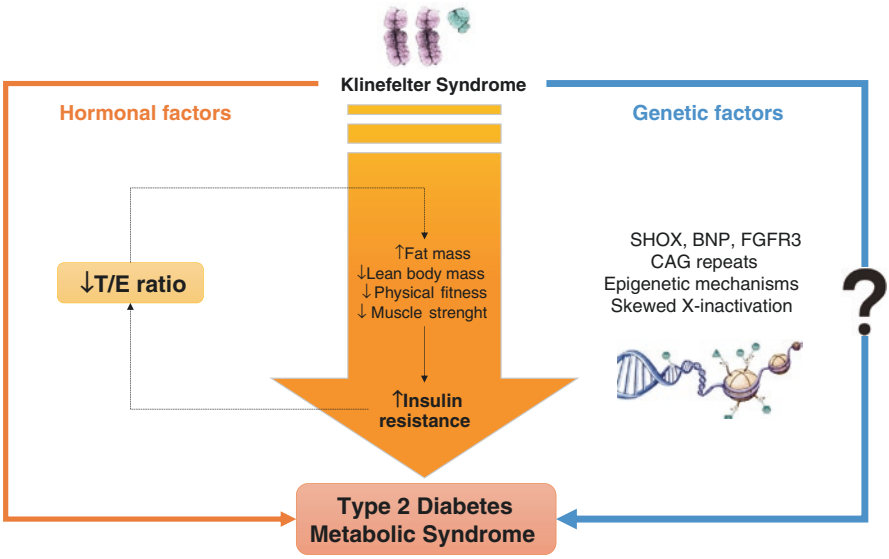

Fig. 18.1 Hypotetical factors involved in metabolic derangements observed in subjects with KS

height with relatively long legs, small and firm testes, and gynecomastia) but also with mild forms that can elude diagnosis throughout life [42, 43]. It should be noted that the AR gene is among those genes that undergo inactivation and may be implicated in the variability of the phenotype observed among KS patients. The length of a stretch containing polyglutamine coding (CAG)*n* repeats plays a key role in this process. The shorter this stretch, the higher is the activity of the receptor, such that androgen effects on clinical phenotype and social characteristics might be modulated by the AR CAGn polymorphism [44]. Selective inactivation of the shorter of the two alleles would be associated with a more severe phenotype and diminished response to TRT [12, 44, 45]. Indeed, a positive correlation between CAGn length of the AR genes and body height, as well as an inverse correlation between CAGn length and the arm-span/body height, has been observed in KS subjects [44, 45]. Whether a different CAGn length may lead to a specific metabolic profile in subjects with KS is still unknown. The AR repeat polymorphism seems to have little influence on the extent of fat mass or its regional distribution in physically active men without KS [46]. However, an inverse relation between the CAGn length and insulin/HOMA-IR index has been demonstrated [47], and independent associations between the CAGn length and cardiovascular risk factors, such as high LDL [48] and low HDL [49], were also shown. Finally, the AR CAG repeat polymorphism plays a role in the response to TRT in males with hypogonadotropic hypogonadism, since shorter AR gene CAG tract length yields greater metabolic improvement following T administration [46, 50]. Thus, a role of the CAGn length and consequently of AR function in the manifestation of the metabolic derangements seen in subjects with KS cannot be excluded (Fig. 18.1).

18.4 Therapy

Despite known connections between T levels and metabolic health, best practices for preventing or treating T2D in KS are not well established. In particular, the effects of TRT on the risk of developing T2D in men with KS, as well as on glycemic control in those with established diabetes, remain poorly understood due to the paucity of randomized controlled trials. A meta-analysis of randomized controlled trials of men with T2D and/or metabolic syndrome found no evidence of TRT on improvements in HbA1c levels [51]. Administration of oral oxandrolone, a non-aromatizable androgen, to 93 prepubertal boys for 2 years was shown to modestly improve factors such as triglycerides, fasting blood glucose, and systolic blood pressure, but only triglycerides were found to be significantly different between groups when adjusted for age and baseline cardiometabolic variables [38].

TRT in hypogonadal KS subjects is able to improve body composition, but has less definite effects in ameliorating lipid and glucose levels [52–54]. TRT in men with low free T leads to an increase in lipid oxidation and a decrease in glucose oxidation, suggesting a shift in substrate partitioning to favor lipid utilization [55]. In hypopituitary men, TRT is associated with higher levels of lipid oxidation [56, 57], while gonadal steroid suppression with a GnRH analogue in healthy young men results in reduced lipid oxidation and diminished energy expenditure [58]. Moreover, in patients with hypogonadotropic hypogonadism, a deterioration of insulin sensitivity may occur within only 14 days after cessation of TRT [59]. However, a worse metabolic profile is still evident in KS subjects after TRT when compared to age-matched controls. Therefore, available evidence does not support TRT of KS patients with the aim of reverting insulin resistance or hyperglycemia, even though TRT-induced changes in body composition and physical fitness may be helpful in this setting [60].

In regard to the most appropriate drugs for the treatment of diabetes or its complications in KS patients, few data are available. However, considering the peculiar susceptibility to fat accumulation (particularly visceral fat) of KS subjects, drugs associated to weight/fat loss should be favored [3, 61].

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Klinefelter Syndrome: Cardiovascular Characteristics

19

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Patients affected by Klinefelter syndrome (KS) have an increased mortality [1] mainly due to overall cardiovascular diseases, with the exception of ischemic heart disease [2]. However, together with thrombophlebitis, venous thrombosis and pulmonary embolism, ischemic heart disease still remains one of the responsible for the higher risk of hospitalization in these patients [3]. Nevertheless, mortality is not just matter of acquired cardiovascular disease: KS has been associated with an increased risk for congenital heart malformations [2, 3].

Due to this concept, echocardiography is mandatory both in children and adults with KS in order to not only detect cardiac abnormalities, but also correct acquired disorders. In the diagnostic process, other than ultrasound examination, genotype is also significant when a cardiac anomaly is suspected. A variety of abnormalities have been described in the commonest form of KS (47, XXY). Transposition of the great arteries [4, 5], patent ductus arteriosus (particularly described in presence of microdeletion of chromosome 22q11.2) [6], partial atrioventricular canal defect and mitral valve malformations [7], tetralogy of Fallot [8], hypertrophic cardiomyopathy [9], and pulmonary arterial hypertension [10] have been described in the classical genotype.

In severe aneuploidies, for example, 49 XXXXY, the most frequently associated cardiac phenotype are tetralogy of Fallot [11] and patent ductus arteriosus [11], but ventricular sept defect [11], ventriculomegaly, atrial septal defect and peripheral pulmonary stenosis [12] are also described. Other complex syndromes, as seen with Down-Klinefelter syndrome (48,XXY,+21), are instead characterized by a different and worse cardiac phenotype which includes anomalies with left-right shunt, obstructive anomalies, and more complex anomalies [13–22]. The few available studies on cardiac damage in KS are quite conflicting. Early studies designed to

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evaluate the presence of left ventricular structural abnormalities reported an increased prevalence of mitral valve prolapse [23, 24]. This condition is associated with chordal rupture [25], mitral regurgitation with an increased risk of sudden death. However, this result was not confirmed in other studies [26].

In respect to acquired disorders, left ventricular structural and functional abnormalities have also been variably described KS. With regard to the systolic phase, systolic velocities and strain rate have been found significantly lower in KS patients. Such alterations could be present particularly in patients with metabolic syndrome. Regarding the diastolic recoil, patients has shown to have reduced E′/A′ ratio, a precocious sign of diastolic impairment. About association with important metabolic parameters, systolic dysfunction is negatively associated with fasting triglyceride levels and truncal body fat, whereas diastolic dysfunction has only been related to truncal body fat [26], underlining the strong relation between metabolic syndrome and left ventricular performance in those patients.

Besides left ventricular morpho-functional alterations, increased cardiovascular risk in KS is also associated to worse exercise performance and subclinical atherosclerosis. Patients have shown to have reduced maximal oxygen consumption, increased intima-media thickness, and chronotropic incompetence (diminished heart rate response to exercise), thus suggesting multiple preclinical cardiovascular alterations that may precede or at least be part of the poor cardiovascular clinical outcome [27]. Endothelial dysfunction has also shown to be involved in KS. KS patients have an absolute lower number of endothelial progenitor cells [28], an independent predictor of atherosclerosis progression [29, 30]. This result was independent from the presence of any cardiovascular risk factor and interestingly from testosterone levels, questioning a genetic-related (and then non-reversible) defect [28].

In conclusion, KS can be associated with a higher risk of congenital cardiac malformations that, despite their frequency has been overestimated, still require an early imaging screening. More importantly, preclinical cardiovascular organ damage consisting in left ventricle systolic and diastolic disfunction, and peripheral endothelial functional alteration, involving the vessel structure and endothelial progenitor cells have also been described in KS. These abnormalities, together with an increased prevalence of metabolic alteration (including diabetes mellitus, dyslipidemia, and metabolic syndrome) [31], may contribute to the increased morbidity and mortality for cardiovascular diseases in KS.

A dedicated cardiovascular work-up, mainly through echocardiographic exam, is suggested in the management of KS patients, aiming to reduce the cardiovascular risk by precocious diagnoses of preclinical and clinical abnormalities.

More research is needed to better describe the cardiovascular complications in KS, possibly elucidating the involved pathophysiological underlying mechanisms and defining the contribution of testosterone replacement in restoring cardiovascular health in KS patients.

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The Thyroid Function

20

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20.1 Introduction

Klinefelter syndrome (KS) is associated with numerous comorbidities: infertility, type 2 diabetes [1, 2], osteoporosis, coagulation and cardiovascular diseases [3, 4], neurological disorders, autoimmune diseases [5, 6].

The prevalence and the etiopathogenesis of thyroid dysfunction in KS are unclear. As far as the alterations of the thyroid function are concerned, the studies are mostly contradictory and most of them are dated and examined on a small number of patients.

20.2 Hyperthyroidism and Klinefelter Syndrome

Using the terms hyperthyroidism and Klinefelter syndrome PubMed™ search, only two published papers were found on this specific topic. In particular, Park JS et al. described a case report of Grave's disease associated with KS, treated with antithyroid medication [7]. A similar case was also previously reported [8]. These two case reports do not allow us to study causal links but only an association.

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20.3 Hypothyroidism and Klinefelter Syndrome

Between the described thyroid abnormalities, the most common is a lack of TSH response to the stimulus with TRH test [9–11].

Bjorn et al. [12] found, in a case-control study, that men with KS had lower FT4 values than controls and FT4 clustered in the lower part of the reference range for the assay. In this study men with KS had no increase in serum TSH or in the TSH/FT4 ratio despite a low FT4; serum FT3 wasn't low in these patients. This patter suggests an inadequate hypothalamic-pituitary control of thyroid function and a possible secondary thyroid insufficiency [12]. They didn't find a correlation between low FT4 and testosterone deficiency [12].

20.4 Association between Klinefelter Syndrome and Thyroid Autoimmune Diseases

The X chromosome contains many genes related to the immune response, including forkhead box P3 (FOXP3), CD40 ligand (CD40L), and Toll-like receptor 7 (TLR7) [13, 14]. There are reports suggesting that people with KS may be at increased risk of some autoimmune diseases, but the evidence is not substantial. A Danish register found that men with KS are more frequently hypothyroid than controls (0.7 vs. 0.005%) but did not have a high prevalence of circulating thyroid insufficiency; in fact, there wasn't an increased prevalence of Hashimoto's thyroiditis [15]. Moreover, in the study of the Panimolle group, the anti-TPO antibody frequency in Klinefelter men was similar to that found in control group and it was similar in testosterone replacement therapy (TRT)-naïve patient and also in previous study [12, 16, 17].

In an English national cohort study of people with KS were selected records in a record-linked dataset of all hospital day cases and inpatient admissions in England, from 1999 to 2011, to identify the occurrence of autoimmune diseases. They found in people with KS a significantly increased risks of Addison's disease (RR 11.7, 95% confidence interval 2.4–34.4), diabetes mellitus type 1 (6.1, 4.4–8.3), multiple sclerosis (4.3, 1.2–11.0), and acquired hypothyroidism (2.7, 1.8–4.0) [18].

20.5 Goiter in Klinefelter Syndrome

The incidental finding at post-mortem of a non-toxic nodular goiter in a 71-year-old man with KS is the only reported data available, back to 1963 [19]. In this report it is mentioned that other authors detected in three KS patients a low iodine intake [20]. It is interesting from a cultural viewpoint of the interpretation of this finding that the authors suggest that at least non-toxic nodular goiter, a disease more characteristically present in females, is more frequent in XXY individuals than in normal males. Clearly, this assumption was not proved, showing lack of any evidence to support this idea.

20.6 Thyroid Function in Klinefelter Syndrome: The KING Study

Balercia et al. [21] performed a recent multicenter case–control work based on the data of the Italian network KING which took into consideration the thyroid function in KS. They evaluated the prevalence of thyroid diseases, the role of 47XXY condition as well as the hypogonadism in thyroid dysregulation in 174 KS patients and 62 non-KS hypogonadal men. In this study, the comparison between KS subjects and non-KS hypogonadal male may contribute to distinguish the impact of the hypogonadism and the genetic origin of thyroid dysfunction in KS.

All the subjects were under testosterone replacement treatment. Testosterone levels were similar in both KS and non-KS, and, in both groups, at the lower end of the normal range. The prevalence of Hashimoto's thyroiditis was 7% in KS and 4% in non-KS. FT4 was significantly lower in KS than in non-KS, while TSH levels were similar. This finding seems to be consistent to previous studies suggesting a hypothalamic–pituitary–thyroid axis dysregulation in KS with hypothyroidism due to hypothalamic-pituitary dysfunction [12]. To verify this hypothesis, the authors calculated TSH index that provides an accurate estimate of the severity of pituitary dysfunction in hypopituitary patients [22]. Surprisingly, TSH index and FT3/FT4 ratio were not statistically different between KS and non-KS with hypopituitarism, living still unclear the origin of lower FT4 levels in KS. Metabolic syndrome and insulin resistance are specific features of KS, and FT3/FT4 ratio has a better predictive power for metabolic syndrome than TSH [22]. They found comparable FT3/FT4 ratio both in KS and in non-KS, suggesting a similar predisposition to increased risk of metabolic syndrome parameters and insulin resistance. The KING study suggests that KS subjects present an impaired production of T4, with an adaptive type II deiodinase activity that allows a normal peripheral circulating amount of FT3 and normal TSH levels. The study does not demonstrate that the alteration of the thyroid function is due to hypogonadism or to changes in the set point of thyrotrophic control.

20.7 Perspectives

Although hypothyroidism in KS doesn't represent a pathology determining a significant reduction in the quality and quantity of life, it would be appropriate to define with further studies the link between the thyroid pathology and KS, studying the entire axis with basal and dynamic dosages extending them to a larger population. Functional studies to clarify the role of deiodinases in the lower FT4 levels seen in KS are also needed. Finally, the identification of the genetic basis of thyroid dysfunction in KS is the key element to better understand the correlation between the extra X chromosome and the endocrine dysregulation.

Author Contributions All authors contributed to the discussion and edited the manuscript before submission.

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Oncological Problems

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Abbreviations

BEP	Bleomycin, etoposide, cisplatin
CI	Confidence interval
CT	Chemotherapy
GCTs	Germ cell tumors
HR	Hazard ratio
KS	Klinefelter's syndrome
MBC	Male breast cancer
SIR	Standardized incidence ratio
TRT	Testosterone replacement treatment

21.1 Introduction

In the first medical report, published by Klinefelter in 1942 [1], no oncological feature was described and it took more than 10 years before the first cases of malignancy were reported in official scientific literature in 1955 [2]. From that report, more than 50 years have passed, oncological problems in patients affected by Klinefelter's syndrome (KS) still represent nowadays a crucial issue for the definition of a correct clinical approach and management of this heterogeneous group of subjects. Nevertheless, most of the evidence available in the field is limited to case reports and only few epidemiological studies have been designed with the aim to

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define the incidence of malignancies among KS patients. It is known that KS is associated to an increase of all-cause mortality, but malignant neoplasms do not appear to have a statistically significant role in this increase. Both the most reliable epidemiological studies so far conducted, respectively in a British [3] and in a Danish [4] population, report an increased mortality among KS group (standard mortality rate 1.5 [1.4–1.7, 95% CI] and hazard ratio 1.4 [1.13–1.74, 95% CI] for all causes). Moreover, the overall incidence of malignancy seems not to be higher than in the general population; however, evidence is compelling about a cancer-specific increase of incidence in this population of patients. These malignancies most frequently described belong to three different groups of tumors: extragonadal germ cell tumors (GCTs), breast cancer and hematological malignancies. The exact mechanism underlying the pathogenesis of these tumors is mostly still unknown, but recent hypothesis includes both the possible role of an aberrant hormonal milieu together with the direct role of the supernumerary X-chromosome and its following consequences on DNA methylation and gene regulation (e.g., gene dosage effects and X-chromosome inactivation pattern; see Chap. 6).

The overall incidence of the cited malignancies is, however, so low that routine screening of an asymptomatic KS male is not yet recommended; it is, however, important for clinicians to be able to recognize their possible presentation in order to guide appropriate investigation and prompt diagnosis and treatment. In this chapter most recent evidence and relevant literature reports will be presented and discussed.

21.2 Germ Cell Tumors

Germ cell tumors (GCTs) are a group of histologically heterogeneous neoplasms deemed to originate from the aberrant proliferation of primordial germ cells during intrauterine life. The typical localization of these tumors are the gonads in both sexes; nevertheless, they can be localized in extra-gonadal site, typically distributed along midline structures: sacro-coccygeal, intra-cranial (pineal or suprasellar region), retroperitoneal and mediastinic localizations being the more frequently observed. Given the increase of incidence of all testicular cancers, GCTs still represent a rare clinical entity. In the male general population, the incidence of GCTs increased over the last decades in both Europe and America, in the latter estimated to be 4.84 per 100,000 men during 1994–1998 period [5, 6], with peak incidence during first 12 months and during adolescence (15–19 years). The first study investigating cancer incidence in a Danish cohort of 696 KS patients estimated a 67-fold increase of risk of development of mediastinum GCTs in this group compared to general population, whereas no case of testicular cancer was described in the same cohort [7]. This finding has been later supported by another study on Danish cohort, in which authors reported a significant higher risk of hospital admission due to a mediastinal tumor (HR 14.2) in KS patients compared to controls [8]. About pediatric population, a recently published work by the Children's Oncology Group described a prevalence of 3% of KS among patients

diagnosed with a GCT in any location (age range: birth to 19 years). In this North American cohort the authors confirmed the significantly higher probability of extra-gonadal localization (70% mediastinal) and calculated an 18.8 risk ratio to develop a GCT among KS males compared to the non-KS males (absolute risk of GCT among KS, 1:2400–6000) [9]. Most of these mediastinic lesions are histologically assigned to non-seminomatous subtype, with teratoma and yolk sac tumors being the most common. The clinical presentation can be various depending on the age of the patient, the size and stage of the tumor and on the concomitant production of human chorionic gonadotropin by the tumor cells at the time of the disease onset. No specific symptom (e.g., cough, dyspnea, chest pain, and fatigue) of thoracic involvement can be part of disease presentation, often leading to radiological investigations. Differential diagnosis of mediastinal masses includes lymphoma, thymoma, thymic carcinoma, and sarcomas; the young age and the anterior position of the mass are highly suggestive of a GCT. An example of radiological aspect of a primary mediastinal lesion is shown in Fig. 21.1. No significant difference in the age of presentation was noted in pediatric populations so far studied [9]; however, the tumor may present in younger ages as precocious pubertal development as described by some case report [10, 11]. Rarely the presentation can occur during adult age [12]. In light of the rarity of the disease, there are no prospective studies comparing different CT schedules or treatment strategies for GCTs. Therefore, their therapeutic approach should be addressed to referral centers. Standard approach relies on first-line medical treatment with bleomycin, etoposide, and cisplatin (BEP) for up to four courses. Recently, high-dose CT regimens have been proposed for patients with poorer prognosis, often requiring stem cell support. After medical treatment, surgical removal of the

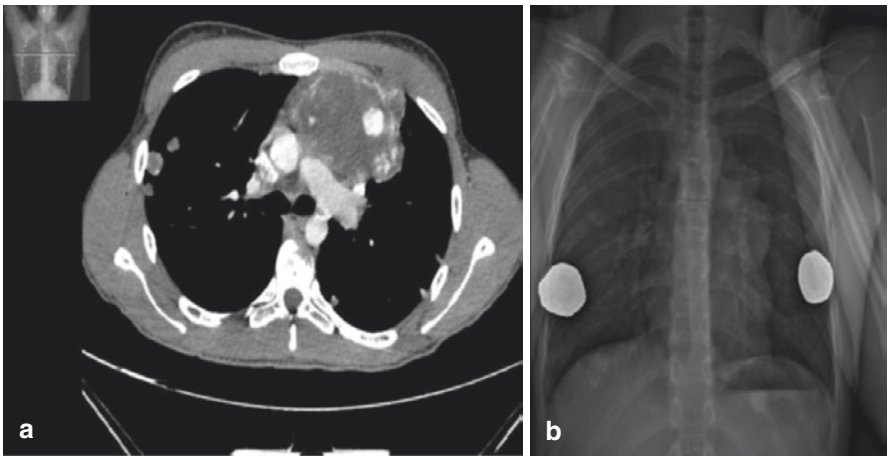

Fig. 21.1 Chest X-ray (**a**) and contrast-enhanced CT scan (**b**) of a 20-year-old patient with hCG-secreting germ cell tumor. Images show a large mediastinal mass (max diameter 66 × 100 mm) with rich intra-lesional vascularization and multiple lung tumor disseminations (max diameter 30 mm)

residual mass is needed to improve life expectancy [13]. Survival rates for primary mediastinic GCTs are reported to be lower than what is observed in intragonadal forms, with long-term survival estimated to be around 50% in the general population [9]. No study aimed to evaluate whether the presence of KS could represent a negative predictive factor on treatment outcome.

21.3 Breast Cancer

Male breast cancer (MBC) is a rare disease with a reported incidence of 1.8 per 100,000 in black men and 1.1 per 100,000 in white men [14]. However, the incidence is slowly rising with a number of new cases in 2019 of 2550 with 480 estimated deaths [15]. The association between MBC and KS was suggested for the first time in 1955 with the clinical description of an 80-year-old man with KS who developed carcinoma of the breast in 1947 [2]. Although it is now recognized that KS patients are at risk for MBC, the magnitude of the relationship remains uncertain due to the lack of large epidemiologic studies with an adequate control group, the selection bias of some studies and the under-recognition of KS in the population basis [16]. One of the first meta-analyses about the epidemiology of MBC reported an increased risk of 30-fold in KS [17]. Later on, another study was conducted retrospectively on 93 unselected male breast cancer patients. The prevalence rate of KS in these males was found to be 7.5%, so the authors concluded that KS patients had a 50-fold increased risk of developing breast cancer [18]. In more recent studies, instead, the reported risk was significantly lower. In 2005 a large British cohort study involving 3518 patients with KS diagnosed between 1959 and 2002 reported a standardized incidence ratio of 19.2 (95% CI, 5.2–49.2) with a higher standardized mortality ratio of 57.8 (95% CI, 18.8–135) compared to general population [3]. More recently, a Swedish cohort study identified 1085 men with KS from the Swedish Hospital Discharge and Outpatient Register that were further linked to the Swedish Cancer Register to examine the standardized incidence ratios (SIRs) of cancer using the general population as reference. They found only one case of MBC with a non-significant SIR and assumed that this finding could be explained by the exclusion of the KS cases identified after MBC [19]. It has been speculated that various conditions observed in KS could explain this association including the altered hormone levels (high estradiol levels with increased *E/T* ratio), the effect of long-term TRT, and the genetic susceptibility linked to the supernumerary X chromosome [20]. Unfortunately, the exact mechanism is not yet completely known. Although gynecomastia could be observed in almost 40% of KS patients [21] and elevated levels of estrogen and progesterone tissue receptors have been found among KS patients with gynecomastia [22], this condition is not considered as a risk factor for MBC [20, 23]. Neither the clinical presentation nor diagnostic and treatment approach differs for KS from general male population. Even if routine screening for MBC is not yet recommended, some authors recommend monthly breast self-examination and yearly examination by the patient's physician in order to obtain an early diagnosis and a subsequent early treatment [16, 24, 25]. The

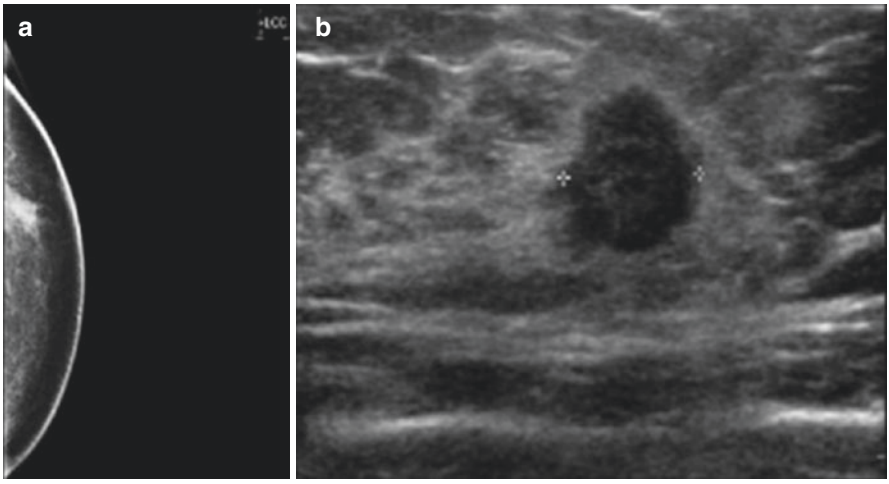

Fig. 21.2 Mammography (**a**) and ultrasound (**b**) of a unilateral palpable breast nodule later diagnosed as a breast cancer in a 41-year-old patient. X-ray image shows an irregular 15 mm opacity located in the upper quadrants. Ultrasound image shows an 11 mm markedly hypoechoic lesion with thick hyperechoic halo and irregular margins

detection of an extra-areolar breast nodule should raise the suspect of MBC and diagnostic assessment should promptly follow. Radiological imaging of an MBC in an adult patient with KS is shown in Fig. 21.2.

21.4 Hematological Cancer

The first case of acute leukemia in a KS patient was reported in 1961 [26]. Since then some other sporadic cases of leukemia and other hematological cancer have been reported, but published literature regarding the risk of hematological malignancies in males with KS is still conflicting and some authors stated about a possible "chance association" [27, 28]. In the British cohort study involving 3518 KS patients mentioned above [3], it was reported a standardized incidence ratio for non-Hodgkin lymphoma of 0.9% (95% CI = 0.3–3.1). Interestingly the risk of non-Hodgkin lymphoma was reported to be higher in patients with more than three sex chromosomes (SIR = 16.2, 95% CI = 3.3–47.4). In the Swedish cohort study on 1085 men with KS [19] the risk of hematological malignancy was increased (SIR = 2.72) and, in particular, non-Hodgkin lymphoma and leukemia showed an increased SIR of 3.02 and 3.62, respectively. The underlying mechanisms of this association need to be better investigated but it could be explained by a higher frequency of gene fusion and/or translocation during cell division due to extra chromosome in the cellular lines [19]. In conclusion more studies would be needed to clarify the pathogenesis between hematological cancer and KS in order to guide monitoring of asymptomatic patients.

Conflict of Interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Fertility Preservation

22

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22.1 Introduction

Klinefelter syndrome (KS) is generally described as a man with tall stature, gynecomastia, sparse body hair, small and firm testes, and hypogonadism resulting in azoospermia. The vast majority of men with non-mosaic KS are azoospermic, while motile sperms in the ejaculate and spontaneous pregnancies from KS fathers have been rarely described [1, 2]. In general, Klinefelter mosaics (47,XXY/46,XY) are less severely affected, therefore the chance of finding sperm in their ejaculate is higher than in non-mosaic cases [3]. For many decades use of donor semen has been the only possible way of becoming a father for KS men. In 1996, Tournaye et al. [4] reported recovery of spermatozoa by testicular sperm extraction (TESE) in a man with azoospermia and KS. One year later, Palermo et al. [5] documented the first pregnancy from a man with KS after TESE followed by oocyte intracytoplamic sperm injection (ICSI). In the last 20 years, hundreds of non-mosaic azoospermic KS men were submitted to TESE and a recent meta-analysis of data from a total of 1248 patients with a mean age of 30.9 ± 5.6 years reported an overall sperm retrieval rate (SRR) per TESE cycle of 43% in non-mosaic azoospermic KS men [6]. Overall, a total of 218 biochemical pregnancies after 410 ICSI cycles were reported [PR = 43% (36, 50)]. Similar results were observed when live birth rate (LBR) per ICSI cycle was analyzed: 211 live births [LBR = 43% (34, 53)] [6]. According to these results, KS men should not be considered anymore infertile and affordability should be placed to maximize their fertility potential. Here we tried to

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answer to questions of critical relevance to improve fertility in azoospermic KS men: is there an ideal age for sperm retrieval or do we know predictive factors for the success of TESE? Is it possible to potentiate spermatogenesis before TESE? Is there a best surgical procedure to perform testicular sperm retrieval? Is TESE harmful for future steroidogenetic potential of KS men? A couple with a KS man submitted to a successful TESE-ICSI should expect a normal live born child?

22.2 Preferential Age for Sperm Retrieval and Predictive Factors for a Success of TESE in KS

Testicular histology in KS features germ cell degeneration starting already in utero, progressing slowly during infancy and early childhood, and accelerating during puberty and adolescence: the final result consists in extensive fibrosis and hyalinization of the seminiferous tubules and hyperplasia of Leydig cells [7–9]. Testicular volume is already reduced in infants and prepubertal boys with KS as compared with similarly aged healthy boys [10, 11], indicating that extension of seminiferous tubules is reduced before puberty. Accordingly, Wikstrom et al. [12] found germ cells in the testes of 50% of peripubertal KS boys. In addition, Wikstrom et al. [13] showed that germ cell differentiation was arrested in spermatogonium or early spermatocyte in KS and the spermatogonia undergo apoptosis instead of entering meiosis at the onset of puberty. Therefore, the chance of finding spermatozoa in the ejaculate would be rare in semen samples from pubertal KS boys [8, 14, 15].

Azoospermic KS men may have residual foci of preserved spermatogenesis and may benefit from assisted reproductive techniques (ART) to father a child [6]. This fostered the hypothesis that testicular spermatozoa might be retrieved in pubertal KS boys before the acceleration phase of germ cell degeneration. Some reports in adolescent KS patients showed success rates similar to that in adult KS patients at age of 15–19 years [16]. However, when age was below 15 years, the success rate decreased to 10% [16, 17]. In a recent large study [8], including more than half of the patients younger than 15 years, spermatozoa could be obtained by TESE in one boy only in the 13–16 years old patient group. The low success rate in the adolescent group was explained by the young age of the patients and it suggested to perform TESE at a later age [8]. It has been postulated that focal spermatogenesis in adult KS men arises from XXY spermatogonia undergoing an erroneous self-renewal with loss of one X chromosome self-correcting to XY spermatogonia [18]. Therefore, early sperm retrieval at adolescent age may even be contra-productive [19].

In recent years, cryopreservation of spermatogonial stem cells (SSCs) has been offered on an experimental basis to immature boys prior to chemo- or radiotherapy with the purpose of being (hypothetically) able to reintroduce the SSCs in the patient's own testis by SSC transplantation. So far in vitro spermatogenesis or SSC transplantation of human SSCs has not been possible, but this technique might become an option in the future since the in vitro differentiation of mouse SSCs to

mature sperm cells has been reported [20, 21]. Cryopreservation of SSCs has been offered experimentally to pubertal KS patients aiming at collecting germ cells before the acceleration phase of seminiferous tubule degeneration. Initial reports, however, concluded that this strategy may be of limited value [12, 22, 23]. Germ cells are lost at a very young age, even before initiation of the fibrotic process in KS testicle. Indeed, very few spermatogonia were detected in testicular tissue biopsies from prepubertal patients with KS, suggesting that preventive testicular stem cell banking remains questionable and fertility preservation strategies are not recommended in KS boys [8].

Some authors have emphasized that KS subjects of a younger adult age have a better chance of positive TESE ([14, 24]) but a recent meta-analysis showed that successful SRR in KS is independent of age [6], suggesting that the focal spermatogenesis in KS testes is not involved in a progressive seminiferous tubule hyalinization although the origin of a scattered preserved spermatogenesis in KS is still hypothetical [18]. Other factors including hormonal levels (FSH, LH, free and total testosterone, SHBG, estradiol, inhibin B, prolactin), testicular volume, testicular ultrasonography, testicular histology, or degree of virilization had no prognostic values for successful SRR in KS patients [3, 14]. Indeed, patients with inhibin B below the detection limit underwent successful TESE [25]. However, a combination of total serum testosterone above 7.5 nmol/l and LH levels below 17.5 U/l resulted in higher SRR by micro (m) TESE in both adolescents and adults with KS [2]. In addition, one study found a positive predictive value of testicular volume, testosterone levels, and response to hCG test for successful TESE [26]. The large meta-analysis by Corona et al. [6] showed that neither testicular volume nor hormonal pattern influenced SRR in KS patients.

22.3 Potentiation of Spermatogenesis Before TESE in KS

In patients already receiving androgen replacement therapy, it has been suggested to discontinue this treatment for at least 6 months prior to mTESE in KS men [27]. Treatment aimed to improve intratesticular testosterone levels has been proposed before TESE in KS men with low baseline testosterone. Medical treatment with aromatase inhibitors, clomiphene citrate, or human chorionic gonadotropin resulting in testosterone level>8.7 nmol/l (250 ng/dl) was associated to a higher chance of sperm retrieval than men who did not respond to treatment [28]. In a non-controlled study, Mehta et al. [29] described a positive SRR at TESE in 7 of 10 KS adolescents and young adults with a baseline level of testosterone <350 ng/dl who received a testosterone supplementation in combination with an aromatase inhibitor therapy for several years (1–5 years) with target serum testosterone level >400 ng/dl. However, data are limited and insufficient to define an ideal treatment of KS men to eventually potentiate spermatogenesis before TESE. Randomized double blind placebo-controlled clinical trials are needed to define whether it is necessary to perform TESE before initiating

testosterone therapy or it is safe to wait until paternity is wished and whether a treatment designed to increase intratesticular testosterone secretion is efficient or not in increasing SRR in KS men.

22.4 Surgical Procedures for Sperm Retrieval in Azoospermic KS Men

Surgical procedures for sperm retrieval include conventional (c) TESE or mTESE. cTESE is based on blind testis biopsies, whereas mTESE is based on microsurgery to identify individual seminiferous tubules with active spermatogenesis [30]. The mTESE technique is considered superior to TESE with respect to maximizing the success rate of sperm retrieval in non-obstructive azoospermia (NOA) [30]. According to an overview on success rates of sperm retrieval in men with KS according to method (cTESE vs. mTESE), better results were obtained with mTESE [3]. A total of 741 patients were included with an average sperm retrieval rate of 50% distributed on 42% by the use of cTESE and 57% by the use of mTESE. Exclusion of 14 mosaics did not change the success rates [3]. A meta-analysis including 1248 patients with a mean age of 30.9 ± 5.6 years and an overall sperm retrieval rate per TESE cycle of 43% in non-mosaic azoospermic KS men, documented no difference by comparing cTESE to mTESE or when a bilateral approach was compared to a unilateral intervention [6]. Authors suggested that the low testis volume in KS might limit the advantages of mTESE in SRR observed in the general population of subjects with NOA [31, 32].

22.5 The Risk of Hypogonadism After Testicular Sperm Extraction in Azoospermic KS Men

Men with KS have both a reduced testicular volume and mean basal testosterone levels already during pubertal age. Considering the whole testis volume, the amount of testicular tissue removed during TESE is higher compared to men with testicular volume within the normal range. This aspect, associated to the lower steroidogenetic ability of testis, rises the risk for inducing or aggravating a hypogonadism in KS men submitted to TESE. Postoperative testicular damage leading to a decreased testicular function has been described as a complication of cTESE in NOA. It has been thought that mTESE compared to cTESE could cause less testicular vascular damage [30, 33, 34], suggesting that mTESE should be preferred to cTESE in case of KS men. However, Ramasamy et al. [33], comparing mTESE and cTESE in men with NOA without KS, reported in both cases a reduced level of serum testosterone at 3–6 months after TESE with a recovery to baseline levels after 12 and 18 months [33]. A recent meta-analysis evaluating the occurrence of hypogonadism after TESE [35] in 15 studies, documented a decreased level of serum testosterone 6 months after TESE, more relevant in KS men compared to NOA, recovering to baseline value 18 months after TESE in

NOA; a partial recovery of testosterone was reported in KS men 26 months after TESE. Although data were not evaluated separately according to the TESE technique [35], most of the KS men were submitted to mTESE in the three evaluated studies [36–38]. In conclusion, available data suggest that KS patients should be monitored endocrinologically after a TESE procedure independently from the surgical technique used.

22.6 The Outcome of TESE-ICSI in KS

The increased possibility of obtaining spermatozoa by TESE to be used for ICSI in KS men open the need to establish whether or not embryos obtained give rise to normal live births. In the meta-analysis of 29 trials by Corona et al. [6] a total number of 218 biochemical pregnancies after 410 ICSI cycles were reported [PR = 43% (36, 50)]. Similar results were observed when LBR per ICSI cycle was analyzed: 211 live births [LBR = 43% (34, 53)]. PR and LBR per ICSI cycle were independent of age, mean testis volume, LH, or total testosterone levels. The use of fresh sperm or of cryopreserved sperm gave no difference in PR and LBR [6], in line with what has been reported in case of TESE-ICSI in NOA [39]. Overall, live children could be obtained in about 16% of men who underwent TESE approach, a number lower than that reported in NOA (25%; [40]), while not different compared to a study on 444 ICSI cycles in NOA patients having a normal karyotype showing a cumulative delivery rate per TESE patient of 13.4% [41]. According to a review by Aksglaede and Juul [3], healthy live born babies without anomalies were conceived after TESE/ICSI from couples including a 47,XXY father. Although conception from KS men is apparently safe, preimplantation genetic diagnosis (PGD) is generally offered to couples with KS who undergo successful TESE-ICSI [3]. Staessen et al. [42] compared the result of PGD in 113 embryos from 20 couples including a KS partner, with 578 embryos from control couples with X-linked disease undergoing PGD for gender determination. Authors found a significantly higher percentage of sex chromosome (13.2% vs. 3.1%) and autosome (15.6% vs. 5.2%) abnormalities in embryos from KS couples as compared with the X-linked couples while no embryo with 47,XXY from KS couples was identified [42]. Overall, 54.0% of the embryos from KS couples were normal with an almost equal sex ratio [42]. A recent large retrospective longitudinal cohort study [24] reported the chance per first TESE to have a live birth of 10.1% (14/138), therefore, lower compared to 16% reported by Corona et al. [6]. Postnatal karyotyping was done in six children born and revealed a normal karyotype. No sex chromosomal abnormalities were found, but karyotypes were not available in all children born. Therefore, data suggest that live births from KS couples submitted to TESE-ICSI generally exhibit a normal karyotype. Although TESE-ICSI gives the chance for an eventually normal live birth in non-mosaic KS men, the cumulative delivery rates per patient initially submitted to TESE is approximately 16% or lower. Therefore, prior to undergoing TESE for sperm recovery, KS patients should be counseled that the majority of them will probably not father a genetically proper child.

22.7 Conclusion

Although the vast majority of men with non-mosaic KS are azoospermic, they should not be considered infertile, since the use of surgical testicular sperm extraction is associated to a positive sperm recovery of approximately 40% per TESE cycle, which is not different to other conditions of NOA. Present knowledge indicates that successful SSR is independent from surgical technique, age of the patient, testicular volume, hormone levels, or degree of virilization of KS men.

Undefined yet is whether it is necessary to perform TESE before initiating testosterone therapy in hypogonadal KS men, or whether it is safe to wait until paternity is wished, by interruption of testosterone treatment and whether a treatment designed to increase intratesticular testosterone secretion is efficient or not in increasing sperm retrieval rates in KS men.

Germ cells degeneration is already severe before and during puberty, therefore explaining the poor results of TESE outcome performed in KS adolescents. This observation suggests a delay of TESE procedure during adulthood. The extensive germ cell degeneration already during infancy and early adolescence in KS explains the poor results of harvesting spermatogonial stem cells from testicular tissue biopsies in prepubertal boys. Accordingly, preventive testicular stem cell banking remains questionable and fertility preservation strategies are not recommended in KS boys.

TESE technique holds a risk for inducing or aggravating hypoandrogenism in KS, independently of the surgical technique, indicating the need of endocrine monitoring after TESE to initiate testosterone treatment if needed.

The outcome of TESE-ICSI in KS men suggests that healthy live born babies with a normal karyotype without anomalies were conceived from couples including a 47,XXY father. However, PGD offered in couples including a KS partner found a high number of embryos with abnormal karyotype in some trials. Although TESE-ICSI gives the chance for an eventually normal live birth in non-mosaic KS men, the cumulative delivery rates per patient submitted to TESE is approximately 16% or lower. Therefore, prior to undergoing TESE for sperm recovery, KS patients should be counseled that the majority of them will probably father a normally genetic child.

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Fertility Versus Infertility

23

Marco Ghezzi, Antonio Aversa, and Andrea Garolla

23.1 Epidemiology

The vast majority of non-mosaic Klinefelter syndrome (KS) subjects (about 90%) suffer from non-obstructive azoospermia and about 10% from subfertility due to severe oligozoospermia [1–4]. In particular, Lanfranco et al. found spermatozoa in the ejaculate of 11 of 131 (8.4%) men with KS [4]. Motile sperm in the ejaculate and even spontaneous pregnancies resulting from KS fathers have been described, although such cases are anectodical [5]. In adolescents with KS aging 12–20 years, sperm has been identified in 70% of ejaculated semen specimens [6, 7]. However, at present, in azoospermic KS patients, thanks to the advances in TEsticular Sperm Extraction (TESE) (see Fig. 23.1) or micro-TESE combined with intracytoplasmic sperm injection (ICSI) techniques, approximately 50% of men with KS will have sperm detected in testicular tissue of which a 50% pregnancy and live birth rate can be expected [7, 9]. It should be emphasized that currently there are no established guidelines for appropriate timing and harvesting technique choices, and only sperm cryopreservation is considered accepted standard of cure [7].

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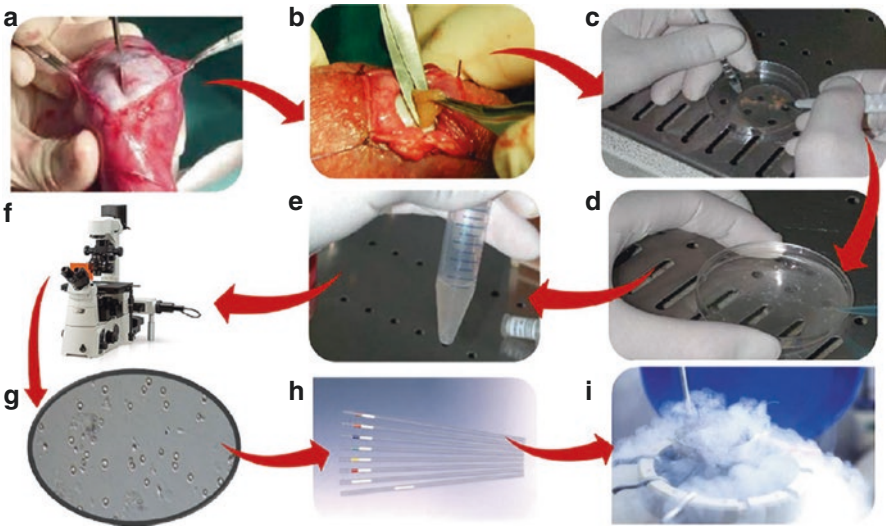

Fig. 23.1 Example of testicular sperm extraction (TESE) and sperm cryopreservation. Panel (**a–i**) show the flow of procedures ranging from surgery to sperm storage: (**a**) transverse incision of the tunica albuginea on the anterior surface of the testis; (**b**) small specimen of a diameter of approximately 5 mm is excised; (**c**) tissue is fragmented in a Petri dish; (**d**) the minced material is transferred in a 15-mL Falcon; (**e**) after centrifugation, the supernatant is discarded and the pellet is resuspended; (**f**) the suspension is checked under an inverted microscope at ×400 magnification for the presence of mature sperm; (**g**) mature sperm together with some erythrocytes; (**h**) the material is transferred and cryopreserved in CBS straws; (**i**) the samples are stored at −196° in a liquid nitrogen tank [8]

23.2 TESE Versus Micro-TESE

KS subjects were considered definitively sterile until the development of TESE-ICSI in the 1990s [10, 11]. For more than 10 years, TESE-ICSI has been proposed for KS subjects and the pregnancy rate is just a little lower than in obstructive azoospermia with normal karyotype: 27% versus 35% [8, 12–14]. The way of performing testicular biopsy varies. Besides the unique biopsies (weight: 250 and 750 mg), multiple biopsies (multisite TESE) (weight 50 mg) have been proposed in the hope to increase the rate of positive sperm retrieval [13, 15].

In 1998 Schlegel develops the technique of microdissection (micro-TESE), which involves the recognition of areas of focal spermatogenesis under an operating microscope [16]. The use of high-power surgical microscope (magnification of ×20– [17] has reduced the amount of testicular tissue needed and resulted in much higher overall sperm retrieval rates (SRRs) [7]. Schlegel reported a significant statistical difference in the overall SRR when comparing patients undergoing standard TESE to those undergoing micro-TESE (45% vs. 63%) and further demonstrated much higher spermatozoa yield from smaller, micro-dissected samples (64,000 vs. 160,000) [7, 15]. Unfortunately, micro-TESE requires highly specialized microsurgical training and close cooperation with

the reproductive endocrinologists and ART team. As such, patients requiring micro-TESE are usually referred to high-volume, specialized infertility centers [7].

Aksglaede and Juul presented an overview of reported SRRs in different studies, with higher values with micro-TESE (mean 57%) than with TESE (mean 42%) in KS subjects [5, 11]. Moreover, a comparative study between testicular microdissection and multiple site TESE in a group of non-obstructive azoospermia (NOA) shows an SRR higher in micro-TESE than multisite TESE (42.9% vs. 35.1%) although this observation did not reach statistical significance [18]. Although in this series KS subjects represent a low percentage, they are comparable in the two groups (16.2% vs. 16.1%). Finally, the success rate in salvage micro-TESE is unaffected by a failed previous multisite TESE, when compared to its use as a primary procedure in a group of NOA with a small number of KS subjects [8, 19].

23.3 Predictive Factors of Sperm Recovery in KS

At present, thanks to the advances in TESE and ICSI techniques, approximately 50% of men with KS will have sperm detected with TESE/micro-TESE, of which a 50% pregnancy and live birth rate can be expected [9]. Reported sperm retrieval rates of about 40% resulted to be independent of several clinical and biochemical parameters, including age, testis volume, and hormonal status at baseline. In addition, the use of retrieved sperm allows live children to be born in ~40% of ICSI cycles meaning a final live birth rate of 16% for the couples who initiated the assisted reproductive techniques. Neither testicular volume nor hormonal pattern is a predictor of sperm recovery. Based on prior data indicating that younger age is a major positive predictive factor for successful sperm retrieval, it has been suggested that fertility preservation may be offered also to prepubertal and adolescent boys with KS. However, the lack of prognostic value of the FSH levels related to the low inhibin B levels (which is almost undetectable during early puberty) in all patients with KS does not allow for the negative feedback on FSH secretion and does not result as determinant for sperm retrieval. The use of any of the non-testosterone-based formulations may be considered in KS men planning on surgical sperm extraction. The selection of this type of therapy and the decision to start should be made on individual basis, following appropriate patient counseling, especially because current clinical evidence for this indication is not well supported by large randomized, placebo-controlled studies.

23.4 Optimal Timing of Sperm Recovery in KS

Currently, there are no established guidelines for appropriate timing and/or harvesting technique choices, and only sperm cryopreservation is considered acceptable for standard cares. Because testicular function decline begins in puberty and worsens in adulthood, the intervention prior to or at the beginning of this decline might yield the same successful sperm retrieval [6].

Therefore, when younger patients are able and willing to provide an ejaculated specimen, they may avoid more invasive surgical interventions in their future. Testicular dissection for sperm harvesting has well-documented negative effects and may result in irreversible scarring and worsening atrophy, as well as further decline in testicular function and resulting decrease in testosterone levels so that cryopreservation of ejaculated samples or testicular tissue samples should be offered to all young, postpubescent KS men who are starting or considering androgen replacement therapy [7]. We are aware that young patients may not be emotionally mature to consider future fertility issues and may not be able or willing to provide an ejaculated semen sample, being afraid of any invasive surgical interventions. However, consistent results from metanalysis [9] show that live children could be obtained in about 16% of subjects who undergo TESE approach. Although no studies evaluating one-to-one comparisons are available, the results of metanalysis are similar to those reported in non-KS subjects with non-obstructive azoospermia [20].

An important consideration in determining the optimal timing of micro-TESE in KS patients is whether fresh or cryopreserved-thawed testicular sperm yields different outcomes with in vitro fertilization (IVF) and ICSI. Although few studies have compared ICSI outcomes between fresh and cryopreserved-thawed testicular spermatozoa from KS patients, we believe that cryopreserved-thawed testicular sperm is a viable option for KS patients who are not actively planning for conception but wish to retain their sperm for future use.

23.5 Fertility in KS Mosaicism

There has been a traditional belief that all KS males who produce spermatozoa in any numbers are XY/XXY mosaics, as XXY cells are meiotically incompetent [21]. However, Foresta et al. demonstrated that there are some 47,XXY germ cells that can go through the meiotic and mitotic processes and mature to become spermatozoa [1]. Other authors have noticed that the presence of sperm in the ejaculate is because the karyotype of the lymphocyte lineage does not predict the chromosomal constitution of testis cells or the presence or absence of spermatogenesis [22]. This was demonstrated by fluorescence in situ hybridization (FISH) analysis of testicular biopsy samples from non-mosaic KS men, which showed that testicular mosaicism occurs (46,XY; 47,XXY) with only the 46,XY cells undergoing meiosis [23]. Thus, it is possible that both processes may be occurring simultaneously within the testes of some men with KS [8]. Future studies should focus on identifying markers to determine which men could expect to have sperm at the time of microsurgical testicular sperm extraction.

In general, men with mosaic KS are reported to be less affected and do seem to be more well-androgenized than their non-mosaic KS counterparts [17, 24, 25]. In fact, men with mosaic KS usually have lower baseline luteinizing hormone levels, lower estradiol levels, and larger mean testicular volumes. A higher proportion of men with mosaic KS have sperm in the ejaculate: about 50% versus

less than 10% among non-mosaic KS. Moreover, among subjects with sperm in the ejaculate, mosaic patients have frequently a higher mean total sperm count [26]. Because the number of men with mosaic KS increases with increased physician awareness and testing, in the future it will be interesting to determine whether the proportion of cells being XXY:XY portends a more severe or less severe phenotype.

In synthesis, men with mosaic KS seem to be more well androgenized than their non-mosaic KS counterparts, both with respect to hormones and sperm presence within the ejaculate.

23.6 Fertility in KS with More Than One Extra X Chromosome

Men with more than two X chromosomes (48,XXXY; 49,XXXXY) are more severely affected than men with the classic 47,XXY karyotype [27]. The phenotype progressively deviates from normality as the number of X chromosomes increases [4]. These men have been shown to function at a lower gonadal level and with more immature behaviors as compared with men with fewer X chromosomes. The presence of two active X chromosomes in animals and hybridoma models is lethal [28]. In females, one X chromosome randomly undergoes inactivation in embryonic tissues [29]. On this basis, it appears that a similar mechanism occurs in men with X chromosome polysomy. DNA sequencing of the X chromosome has identified over 1000 genes involved in a variety of physiologic and pathologic disease states, particularly in brain and testicular function [30, 31]. Genes located in the X chromosome inactivation center are responsible for the detection of an additional X chromosome and the subsequent activation of the X chromosome inactive-specific transcript promoter [32]. In turn, this promoter binds to the supernumerary X chromosome and turns off gene transcription. In men having an extra X chromosome, the presence of the non-inactivated genes from the extra X-chromosome is thought to be the source of much of the pathology, although the exact mechanism remains unknown. Given the theory that much of the pathology of KS is due to the presence of the non-inactivated extra genes from the X-chromosome, it would be a rational extension that men with more than an extra X chromosome would have a worse general and testicular phenotype.

23.7 Future Perspectives

Future research is needed to expand our understanding of the endocrinological impact of some fertility markers, that is, AMH and inhibin B, at various stages in life in males with 47,XXY, specifically in school-age and prepubescent children [33]. This is because research has shown that boys with 47,XXY enter puberty at the expected age but may experience a delay of primary and secondary sexual features. There is a need, therefore, to explore the hormone replacement therapy timing and its impact on sperm recovery as well. Nevertheless, the impact of testicular

neoplasms, that is, Leydig cell tumors or micro-calcifications and cysts/nodules, seems to be low; it should always be investigated with ultrasound techniques or magnetic resonance imaging (MRI) in order to prevent accidental lesions.

23.8 Conclusions

The vast majority of males with 47,XXY are usually azoospermic, but the chances of fathering a child by the use of ART are increasingly encouraging. An average sperm retrieval rate of 50%, ranging from an average of 42% by the use of TESE to an average of 57% by the use of micro-TESE, is reported in the literature [5]. Although approximately half of cases are successful in retrieving sperm, the reported number of live-born children of couples with KS is still limited. Spermatozoa may occasionally be found in the ejaculate, and we therefore recommend always performing analysis of ejaculated semen before considering TESE/micro-TESE.

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Psychological Features

24

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24.1 Language Problems

Defects in language formulation, literacy, and social skills are common in males with Klinefelter syndrome (KS) [1–3]. However, the interpretation of the data emerged is challenging due to the variable methodologies used in the studies, as well as the largely reduced sample sizes. The limited information relates in particular to disturbances in speech. Children with KS are late in making their first stammering and in acquiring sound. They show difficulties in the period of breastfeeding, in the coordination of sucking and in the organization of the skeletal muscle [4, 5]. The aspects of oral motor function during childhood or adolescence have not been studied in depth. The same could be said about the noise in the acquisition of sound.

Over the course of development, language skills of individuals with KS appear to be lower than overall cognitive performance [2]. In general, reading and writing difficulties are closely related to language disorders in early childhood [6]. The defects emerge in the early school years and seem to get worse with age. During late adolescence, males with KS exhibit skills of four or five levels lower than their peers in different academic areas [7]. Some authors have deduced that literacy and language challenges faced by children with KS may be related to short-term auditory memory disorders [8]. However, further studies are needed that examine the

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association between spoken language, literacy, and linguistic memory, that is, the ability to apply operational memory to content and/or linguistic structure. Ross et al. also found that pragmatics is the key form of communication compared to other language skills in KS cases [9]. Some authors hypothesized that verbal memory deficits constituted a key factor underlying linguistic problems in KS [10]. Individuals with KS are at risk of various neuropsychological and communication disorders; however, what emerges on the basis of significantly reduced evidence is that further research is needed to delineate the nature of these disorders [11].

A recent study [12] aimed to characterize the broad communicative phenotype of individuals with KS in a cohort of individuals from preschool to adolescence. The results indicate that phonological problems are particularly characteristic in the sample and that for many KS boys (35%) these problems persisted in adolescence. This has a crucial meaning for those who present KS because phonological errors, in particular atypical or disordered phonological profiles, put children at greater risk of subsequent literacy and academic difficulties [13, 14]. Thus, it is possible that early treatment of speech sonic errors may help moderate subsequent literacy deficits observed in subjects with KS. Previous studies have suggested that expressive language is compromised more frequently and to a greater extent than receptive language [2]. Early detection and intervention of phonological errors can reduce the risk of subsequent language and literacy challenges and optimize academic and ultimately social and behavioral difficulties later in life.

24.2 Negative Body Image

Several studies show that boys and men with KS report a less positive body image and a high dissatisfaction with certain body parts such as the chest and genitals with reference to gynecomastia and low testicular volume [15]. This leads to high levels of anxiety related to experiences of anxiety related to identity, social isolation, side effects of hormone therapy and/or negative social experiences, the lack of openness to being able to speak freely about one's illness. Klinefelter subjects need to deal with their perceived body dismorphism. This problem may be managed with a cognitive elaboration of dysfunctional thoughts associated with the body image. Better self-assurance can reduce stigma, support positive relational (and sexual) experiences, and provide a sense of control on the disease. In a recent study on the quality of life of adolescent and adult KS subjects, it emerged that patients often feel clumsy and uncomfortable with their body, in conjunction with language disorders; it is considered a source of social challenge. In addition, many subjects reported that they are/or have been bullied because of their physical appearance [15]. The psychological implications of this fact are believed to persist for a long time after the end of the bullying period [16, 17]. The resulting dissatisfaction with the body image positively correlates with anxiety and depression and contrary with self-assurance [18]. The severity of the phenotype (such as the presence of gynecomastia) is among the major predictors of the psychosocial condition of males with SK [15, 19]. In general, it could be said that living in a healthy way with your body

image represents an additional difficulty in the life of these people. The Klinefelter subject needs to compare, elaborate, integrate, and overcome any kind of difficulty in relation to the diversity of his or her own body compared to that of peers.

24.3 Cognitive Functioning and Social-Cognition-Related Difficulties

Subjects with Klinefelter syndrome have been for long time under-diagnosed. This is true especially for the youngest age [20], maybe since this syndrome, which is generally associated with fertility problems, was brought in the light only when the coupled men decided to have a child. Nowadays, many literature evidences highlight, on the contrary, the necessity to perform an early diagnosis, which must consider also the evaluation of Klinefelter syndrome-related problems, such as the cognitive impairment, the language and learning difficulties, and the specific impairment in the Social cognition. All these aspects make the patient's quality of life worsened in different areas.

What is known about the cognitive impairment in Klinefelter subjects is that they present difficulties in a specific area, named executive functioning. Executive functioning refers to a series of cognitive abilities, which permit to a person to organize the information and plan the behaviors in order to resolve some problems. The cognitive abilities implicated in executive functions are sustained attention, working memory, inhibition of irrelevant information, and flexible thought. Although literature evidence shows that Klinefelter subjects present several difficulties in executive functioning, it was supposed that these problems might be mediated by linguistic difficulties. In a recent study, Skakkebaek et al. [21] found that the difficulties in executive functioning were mediated by intelligence quotient (IQ) and social skills.

A central aspect among the executive functions is the cognitive flexibility [22]. Cognitive flexibility, or attentional flexibility, is a specific aspect belonging to the executive functioning macro-area. This ability is strictly related to social adaptation. Cognitive flexibility is related to the capacity to disengage attention away from one source and then move and reengage it to another [23] Measured during clinical psychological practice with specific standardized tools, cognitive flexibility appears to be often impaired. Specifically, in a study of van Rijn and colleagues [22], the authors found that Klinefelter patients have the tendency in persevering with the use of a logic strategy requested for the resolution of the questions at the Wisconsin Card Sorting Test [24]. This perseverance does not give the possibility to discover and adopt more useful strategy for the resolution of the logic question. In addition, poor flexibility is related to difficulties in regulation and modulation of behavior, in the perseverative or rigid behavior in complex situations or novel-like social situations. Hence, cognitive flexibility may imply social adaptive problems which worsen the patient's quality of life. In addition, cognitive flexibility is also associated to attentional capacity. In Klinefelter subjects the presence of attention-deficit/hyperactivity disorder (ADHD) was found in about 60% of cases [25].

Cognitive impairment, associated with verbal difficulties, may partly explain why Klinefelter subjects show difficulties also in social cognition. Social cognition is an ability, which is necessary in the comprehension of several social conditions. Specifically, it is implicated in the perception, comprehension, and expression of the information in a social context. As for the social interaction, social cognition necessitates the integrity in spoken (listening and speaking) and written (reading and writing) language processing [26]. In this regard, Klinefelter subjects show some similarities with subjects with a diagnosis of autism spectrum disorder (ASD) in the difficulty to elaborate social information. All information from the social context must be decoded considering that the others may have different beliefs from ours. This is possible only if the subjects take in mind the other point of view [27], and if they can adopt a flexible thought, together with a good language competence.

In the field of the neurocognitive studies, searching for a cause of social difficulties among ASD subjects, researchers have focused the attention on the elaboration of the emotions from the human faces [28]. Little attention has been instead paid to genetic conditions which may be related to the same social difficulties. An example is the case of Klinefelter subjects, for which exists a substantial risk for social difficulties, due to the evidence of a tendency to develop in a social context introversion, anxiety, and impulsivity [29, 30]. These difficulties may be partly related to the incapacity to perceive in the others the experienced emotions, and to adequate their own emotions in a specific social context. At this purpose, some researchers have found that Klinefelter subjects have the tendency to be less speed and accurate in the tasks evaluating the processing of visuo-spatial patterns, faces, and facial emotions [31].

The study of social cognition in individuals with a diagnosis of Klinefelter syndrome is useful for different aspects. First, the evaluation of a social impairment may help the clinicians in developing specific psycho-educational interventions aiming to improve social cognition in these patients. These interventions might contribute to ameliorate the global psycho-social wellbeing of Klinefelter subjects. Second, the understanding of the mechanisms and pathways associated to social functioning in a different scientific context, in which they are typically studied, that is, the ASD, gives the opportunity to study social cognition beyond the specific neurocognitive diagnoses.

24.4 Psychopathological Vulnerability

Klinefelter syndrome (KS) is described as being associated with a greater psychopathological vulnerability [25, 32–37] and an increased risk of hospitalization for psychiatric disorders (hazard ratio: 3.65), as reported by a Danish study on a large population of subjects with KS (n. 832) compared to a control group [38]. In particular, several studies report an increased incidence of schizophrenia spectrum disorder [25, 35, 38–47], conduct disorders, anxiety, and depression [33, 48, 49], as well as deficits in attention and impulse control [50] in subjects with KS. Furthermore, a higher prevalence for autistic traits and attention hyperactivity disorders seems to

be more commonly present [25, 38–40, 44, 46, 47]. Despite the wide spectrum of morbidities described among KS patients, the most common ones are anxiety and depression, with 18% of KS subjects suffering from generalized anxiety and a range between 19 and 24% from mood disorders [25].

The relation between KS and associated psychiatric symptoms has not yet been completely explained by current research. Scientific literature underlines the possible role played by genes that are overexpressed in the brains of XXY males as being relevant in the development of psychiatric symptoms [35]. In particular, regarding mood disorders, it is not clear if depressive symptoms may be related to the genotype alterations of KS or may be secondary to the physical and psychological features of the KS [51]. As described by Van Rijn et al. [46], patients with KS seem to be characterized by increased emotional arousal and by difficulty in the identification and verbalization of emotions. To this regard, an altered functioning in the emotional brain, such as the amygdala and other limbic structures, is reported in KS [52–56]. Furthermore, Skakkebaek et al. [49] stress the role played not only by personality traits, but in particular by neuroticism and deficits in social engagement (one's general social skills and experiences, including interpersonal attention and communication) as important factors that may be involved in explaining the increased risk for mental health vulnerability. In a recent multicentric cross-sectional study aimed to evaluate psychiatric symptoms among persons with DSD, subjects reported feelings of shame and challenges in developing a positive self-esteem and body image [57].

The complex psychopathological presentation that subjects with DSD may present underlines the need for multidisciplinary care that involves specialized mental health professionals in order not only to guarantee support and prevent associated psychopathologies, but also to help reach psychological wellbeing.

24.5 The Communication of the Diagnosis

The discovery of infertility is one of the main sources of concern for Klinefelter patients; the impossibility of having biological children is experienced by these subjects as a profound loss; and the acquisition of such news often produces reactions of devastation and loss of control [15]. These reactions are additional to those already identified in the literature on male infertility in the absence of SK: feeling of anguish, stigma, loss of control, low self-esteem, guilt, and anxiety [58, 59]. It has been observed that the pain and anxiety caused by this condition of sterility are not only experienced by the patient but also intensely experienced by his parents [60]. In many cases, especially when the diagnosis of SK is made in the prenatal or developmental period (childhood/adolescence), parents express concern about their child's gender identity, orientation, and sexual function [60, 61].

KS concerns a condition that accompanies the patient throughout his life span; for this reason, its discovery becomes a crucial moment in the existence of the individual. He will have to accept and learn to live with an indissoluble part of him. Therefore, presenting the disease in the most correct way becomes a challenge for

clinicians and a good starting point for promoting understanding and acceptance in patients. To date, there is no single strategy regarding the communication of the diagnosis of KS. In fact the usual way and type of information provided regarding KS vary greatly in relation to the health specialist who is consulted [62], and this unfortunately translates many times into negative experiences for those who turn to it. The need to find guidelines in KS communication is reinforced by the fact that there is hardly anything updated in the literature on the subject.

To date, the finding of genetic alteration XXY can occur on several occasions; however, the results of the research suggest that only a low percentage of the affected subjects is identified: in Europe it is estimated that even 75% of cases are never diagnosed [63] mainly due to the great variability of the phenotype [61]. Although prenatal screening tests have made significant progress in recent years, they represent only 25% of the findings [64]. This means that there is a large portion of the population with KS who only become aware of their condition years after birth. Exemplary is a clinical case reported by Bhartia and Ramachandran [65] in which a patient who was diagnosed with the XXY genetic condition at the age of 14 was not told for 46 years. Although it is not always possible to reach such extreme situations, the diagnosis of KS at a more or less advanced age is a real phenomenon. Often these people go unsuspecting to the doctors because of the implications that SK can create for their health or they can turn to the experts only when they are unable to have children, identifying the cause of this in other issues related to SK such as the infertility and azoospermia [66]. It is important to underline that it is highly preferable to diagnose this condition as soon as possible because doing so would allow for early identification of possible language and learning disorders and would also ensure better monitoring and prevention of hypogonadism and its effects [67].

In communicating the outcome of the karyotype analysis, the doctor takes on the task of notifying the presence of a genetic disease which, in addition to displacing the patient for normal concerns regarding his own health, can provoke a deep emotional experience in him: it is imaginable to understand how such news can conflict with the identity that the patient has created over the years up to that moment and destabilize him. In the case previously reported, the patient recalls that the moment of communication of the diagnosis was characterized by the flow of many negative emotions, from asking many questions, from feeling one's life as a lie, and from losing one's sexual identity. The delicacy with which the doctor communicates the discovery of KS can certainly prevent the explosion of negative emotions that normally characterize this moment. The clinician must provide a complete description of the disease and related aspects based on updated information, answer possible doubts such as those concerning sexual identity, and agree with the patient an action plan to intervene on the most urgent health problems of the subject. It also seems indispensable to give, in a moment of discouragement such as that of the discovery of SK, a psychological support capable of helping the patient to talk and discuss about his/her feelings related to such news. The aim is to accept the diagnosis and promote mental health. The communication of information about the disease and the way in which it is dispensed is of fundamental

importance so that incomplete and totally or partially untruthful information is not given with the risk that patients and family members come to imagine disease scenarios that are scarce representation in reality taking on anxieties and worries that are difficult to sustain [68].

In conclusion, it can be said that the communication of the diagnosis is an emotional as well as an informative event and that an exhaustive communication is fundamental for the acceptance of the disease but does not protect from the possibility of developing fears and social behaviors of defense. The patient should benefit from a psychological support when he highlights a psycho-sexological discomfort due to the KS diagnosis [69]. The assessment of any emotional, sexual, relational and affective problem already in the diagnostic phase may give the possibility to the patient and the couple to manage, through a specific psycho-sexological counselling, their difficulties, in order to prevent a general impairment in the Quality of Life (QoL).

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Sexual Function

25

Giulia Rastrelli and Mario Maggi

25.1 Introduction

Testosterone (T) is a key hormone for sexual behavior in either men or women. In males, it plays a pivotal role at each level of sexual function. In fact, T is considered the fuel for sexual desire; it is deeply involved in neurovascular events leading to penile erection and it plays a role in ejaculation.

Klinefelter's syndrome (KS), besides several clinical features that could occur with a wide range of interpersonal variability, is almost invariably characterized by testicular dysfunction. Although testicular dysfunction has inhomogeneity in its presentation, it eventually arises in almost all cases. This results, on one hand, in azoospermia, and, on the other hand, in steroidogenesis failure with lower circulating T levels.

Despite the high prevalence of hypogonadism in KS, surprisingly, sexual function in this category of patients has been scarcely investigated.

25.2 Brain Areas Correlated with Sexual Function

Sexual behavior is regulated by a complex neurological network that is still only partially understood. This is because none of the components is "purely effective" but it is rather involved in several functions, which include emotions and sexual

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cognition [1]. Among the most pivotal areas of this network, the amygdala, the nucleus accumbens, and the hypothalamus should be mentioned as subcortical regions, whereas the orbitofrontal cortex, the anterior cingulate cortex, and the ventromedial prefrontal cortex are the major cortical regions [2]. The amygdala is particularly important since it is richly connected to other brain regions involved in emotions and it represents the coordinating nucleus that integrates information and processes their emotional significance eventually leading to the conscious awareness of feelings and drives, including sexual desire. This role is supported by animal and human studies showing that amygdala is powerfully activated in response to sexual stimuli [3] and during mating [4]. In addition, the amygdala is deeply involved in reward circuitries [5] and reward is pivotal for sexual desire and maintenance of sexual behavior.

Not only amygdala activation, as detected by magnetic resonance imaging (MRI) or electrophysiology studies, has been associated with the aforementioned behaviors but also its volume has been shown to be related to these functions. In fact, amygdala volume in humans positively correlates with the ability to discriminate the emotions shown by pictures of subjects with different facial expressions [6]. Moreover, amygdala volume is associated with sexual functioning in several animal models [7] and this has been shown also in human subjects undergoing neurosurgery for intractable epilepsy [8].

25.3 Androgen Dependence of Brain Area Connected with Sexual Function and Relative Findings in KS Men

It is interesting to note that amygdala is a cerebral area subjected to gender dimorphism in both dimension and function, and this difference is thought to be responsible for several gender-oriented behaviors, including social, parental, and sexual ones. Indeed, T is involved in the determination of this dimorphism. In fact, amygdala largely expresses the androgen receptor and it is demonstrated that the intrauterine exposure to androgen in female animals is associated with masculinized brain and behavior [9]. Further modifications occur during puberty when a direct relationship between amygdala volume and serum T levels is found together with a parallel progression of amygdala growth and Tanner stages [9]. The impact of androgens is still evident in young adulthood when T has been demonstrated to be responsible for the establishment and reinforcement of the connections between the amygdala and frontal areas [9].

As compared with subjects with normal chromosome pattern, KS patients exhibit an increased prevalence of abnormalities, such as cryptorchidism, microphallus, or hypospadias, which suggest a reduced exposition to androgens during fetal life. This is in line with the finding of Leydig cells hyperplasia and degeneration of seminiferous tubules that are already detectable in KS fetuses. However, the measurement of androgens in amniotic fluid from KS fetuses have never yielded significant differences from normal pregnancies [10]. This weakens the hypothesis that very early onset hypogonadism could occur in KS patients. More information is

available concerning pubertal development and hormonal trends during puberty. In KS patients, pubertal development initiates normally with testis enlargement occurring with a timing similar to normal peers. However, after initial development, testis growth stops and even a regression is observed so that most KS patients have a testicular volume below 4 mL. An increase in follicle-stimulating hormone (FSH) parallels the testicular involution whereas the decline in T with increased luteinizing hormone (LH) could be contemporary or later during adulthood.

According to the aforementioned description of androgen dependence of the amygdala, whenever hypogonadism verifies in KS patients, it has the potential to alter the normal development of brain structures important for emotion processing and sexual behavior.

Men with KS present some differences in brain structure and function in comparison with men with a normal karyotype. In particular, in KS patients a reduction in the volume of the amygdala, as well as other regions involved in emotional processing and sexuality, has been observed through MRI studies [11]. Besides volume, a decreased density in gray matter and in cortical thickness have been shown in KS men since childhood [11]. Consistently with the aforementioned androgen sensitivity of brain regions involved in emotion and sexuality, the characteristics of the amygdala in KS men are partially corrected by T treatment [12]. However, though T replacement in KS men modifies the brain leading to a phenotype more similar to men with normal karyotype, it does not completely fill this gap. This leaves room to hypothesize that factors other than T can be involved in the determination of differences in brain areas that dominate sexual function. Genetics is an obvious factor to evaluate. A few years ago, a study on the role of genetic factors on neuropsychological phenotype in 73 KS men and 73 controls has been conducted [13]. In this study, it has been shown that skewed chromosomal inactivation in KS patients is associated with decreased gray matter volume. However, this did not translate into any differences in social skills or psychological distress.

The peculiarities of brain regions involved in sexual behavior certainly leave room to the hypothesis that KS men could have an abnormal sexual function. However, brain structure is not the only reason for hypothesizing that KS men could have impairments in sexuality. In fact, besides low serum T, they are more prone to develop metabolic derangements including obesity and glycolipid alterations. These are treated in detail in different sections of this book. However, a brief discussion focused on the impact on sexual function will be provided.

25.4 Metabolic Impairment in KS Men and Impact on Sexual Function

KS men have a four- to sixfold increased risk of having metabolic syndrome. The higher frequency is observed since younger age, with a prevalence among prepubertal boys of 8% and further 36% having two criteria of metabolic syndrome. The pathogenic mechanism of this metabolic derangement is still unclear. There is evidence that low T levels in men predispose to the development of several metabolic

derangements and the metabolic syndrome itself [14]. This could represent a possible mechanism for KS subject. However, the relationship between serum T levels and metabolic derangements in KS patients is controversial [15]. On the other hand, T treatment is not associated with a consistent improvement in glucose and lipid profile and even a worsening is described in some studies [15]. At present, most of the data on this topic results from observational studies whereas randomized clinical trials are scanty. For this reason, it is not possible to draw conclusions on the possible causal role of T treatment on metabolic derangements in KS men.

Even better documented is the effect of metabolic syndrome—as well as other conditions due to insulin resistance—on the hypothalamus-pituitary-gonadal axis in men. In particular, metabolic syndrome is associated with an almost doubled prevalence of hypogonadism [16]. Typically, insulin resistance and its cognate metabolic alterations lead to the development of secondary hypogonadotropic hypogonadism [17] characterized by LH levels inappropriately normal for circulating T, which is the result of an impaired ability of the hypothalamus and pituitary to react to T decline. It could be supposed that metabolic syndrome in KS patients could represent an aggravating factor for hypogonadism by negatively interfering with an initial and transient period of compensation of hypogonadism, thus resulting in earlier development of the overt condition.

Hence, metabolic derangements can impair sexual function through a worsening in the gonadal status. However, metabolic diseases have also a direct detrimental effect on male sexual function. Erectile function is impaired by obesity and, in particular, visceral obesity is associated with a significant impairment in erection independently of age and T levels [18]. In accordance with the independence of aging and hormone levels, visceral obesity is associated with significantly worse penile blood flow as assessed by penile color Doppler ultrasound (PCDU), thus suggesting an atherogenic component of the symptom in obese men [18]. The detrimental impact of diabetes mellitus on erectile function, as part of the spectrum of the arterial disease, is well known. However, it is important to mention that not only diabetes mellitus but also hyperglycemic conditions, such as impaired fasting glucose, are associated with impaired erectile function and lower peak systolic velocity at PCDU, independently of age, serum T, obesity, and other possible confounders [19]. Similar results were found also for triglycerides, showing that, independently of T levels, age, obesity, and other confounders, their serum levels are positively associated with a worse erectile function and penile blood flow [20], again suggesting that high triglycerides can cause atherogenic erectile dysfunction (ED).

25.5 Sexual Function in Men with Klinefelter's Syndrome

The paragraphs above describe the pathophysiologic characteristics of KS men that can favor the development of sexual dysfunctions. Based on the rationale provided above, an impairment may potentially occur at any phase of the sexual response from the appetitive to the consummative. Quite surprisingly, sexual function in KS men has been scarcely investigated so far. A review on this topic has been published

in 2010 including three clinical studies [11]. Since then, only four further studies have been published. This section summarizes the results and main conclusions of the available studies, whose main characteristics are reported in Table 25.1.

The first available study, which evaluated sexual function in KS men and the effect of T therapy, was published in 1982 [21]. It is a small randomized placebo-controlled clinical trial that evaluated four KS men randomly assigned to receive oral T undecanoate 160 mg daily or placebo for 8 weeks with a crossover design. At baseline, KS men had average T levels in the range of moderate hypogonadism and only one out of four men reported ED. No other relevant sexual complaints were reported. After T treatment, a significant increase in sexual thoughts and sexual excitement was observed, supporting a beneficial role of T treatment in these men. After this small randomized clinical trial, several years passed by before new data on sexual function in KS men were published. In the study published in 1997 by Yoshida and colleagues [22], 40 KS patients evaluated for infertility were compared with 55 non-azoospermic infertile men with normal karyotype. No metabolic data were available for either of the groups whereas T levels were measured only in KS men, with 52.5% of patients being hypogonadal. The prevalence of impaired sexual desire or ED in KS men was quite low (10% and 2.5%, respectively). Complaints about ejaculatory latency time (either premature or delayed) were reported by 57.5% of KS men. About 20% of KS men reported a decreased orgasmic function and 42.5% a decreased semen volume. However, none of the aforementioned symptoms was significantly more frequent in KS than in 46XY controls. Corona et al. [23] considered a cohort of 1386 men consulting for sexual dysfunction. Among these, 23 were non-mosaic 47XXY, which translates into a prevalence of 1.7% that is more than eightfold higher than that reported in the general population [15]. When comparing KS men with the rest of the sample, they reported more often low sexual desire and guilt with masturbation, as well as reduced ejaculate volume and later andrological referral. Interestingly, when comparing the 23 KS men with 92 controls matched for T levels—as well as age and smoking habits—in order to rule out the impact of T on sexual symptoms, the aforementioned differences were not confirmed. This suggests that sexual symptoms in KS men are due to androgen deficiency rather than being an intrinsic characteristic of the syndrome. In a following study [24], ED and PCDU parameters were assessed in a group of 15 non-mosaic 47XXY KS men and 20 acquired hypergonadotropic hypogonadal men with a prepubertal onset, before and after T treatment. The groups did not differ in glycolipid profile and T levels at baseline and follow-up. Neither the International Index of Erectile Function (IIEF-5) score nor PCDU parameters were different at baseline. T treatment resulted in an improvement in sexual function and penile blood flow in either of the groups, although it was significantly greater in the non-genetic hypogonadal group than in KS men. However, it should be underlined that this study is not a randomized trial and it was not specifically designed and powered for sexual outcome. These results confirm that sexual symptoms in KS men are dependent mainly upon hypogonadism. This is further shown in a following study performed on men consulting a clinic for infertility [25]. Fifty-three non-mosaic 47XXY KS men were compared with 75 46XY controls for sexual symptoms and

Table 25.1 Characteristics of the studies assessing sexual function in men with Klinefelter's syndrome

	Study design	Reason for andrological referral	Assessment of sexual function	KS/ controls (*N*)	Erectile function	Sexual desire	Ejaculatory function	Ejaculate volume	Frequency of intercourse	Orgasmic function
Wu et al. [21]	• RCT	• Infertility	Self-rating	4/0	• One man with ED (25%)	• Frequency of sexual thoughts weekly, 2.5 ± 0.4	• Frequency of ejaculation weekly, 2.6 ± 0.5	Not assessed	• Frequency of intercourse weekly, 4.5 ± 1.4	Not assessed
	• T vs. placebo for 8 months	• Sexual dysfunction			• Not assessed as outcome of T therapy.	• After treatment TTh: 2.6 ± 0.5	• After treatment TTh: 3.8 ± 1.8		• After treatment TTh: 5.9 ± 2.6	
	• Crossover design					Placebo: 1.8 ± 0.1 $p < 0.025$	Placebo: 2.3 ± 0.8 NS		Placebo: 3.9 ± 1.3 $p < 0.10$	
Yoshida et al. [22]	• Observational	• Infertility	Not validated questionnaire	40/55	• ED prevalence, 2.5%	• Prevalence of reduction, 10%	• Prevalence of altered timing, 57.5%	• Prevalence of reduction, 42.5%	Frequency of intercourse weekly, 4.4 ± 2.8	• Prevalence of reduction, 20%
	• Cross-sectional				• No significant difference vs. controls	• No significant difference vs. controls	• No significant difference vs. controls	• No significant difference vs. controls		• No significant difference vs. controls
Corona et al. [23]	• Observational	• Sexual dysfunction	Validated structured interview	23/1363	• ED prevalence, 22.7%	• Prevalence of reduction, 60.9%	• Prevalence of PE, 9.5%)	• Prevalence of reduction, 61.9%	• Prevalence of reduction (<2/month), 50.0%	Not assessed
	• Cross-sectional				• No significant difference vs. controls	• Significantly more frequent than in controls	• No significant difference in PE vs. controls • Prevalence of DE, 9.5% • No significant difference in DE vs. controls	• Significantly more frequent than in controls	• No significant difference vs. controls	

Condorelli et al. [24]	• Case-control • Observational before-after therapy	• Andrological counseling	Validated questionnaire	15/20	• IIEF-5 score, 15.0 ± 3.0 • After TTh KS: 20.0 ± 4.0 Controls: 24.0 ± 6.0	Not assessed	Not assessed	Not assessed	Not assessed	Not assessed
El Bardisi et al. [25]	• Case-control • Retrospective revision of charts for KS • Collection of data for newly enrolled controls	• Infertility	Validated questionnaire	53/75	• ED prevalence, 18.9% • No significant difference vs. controls	• Prevalence of reduction, 54.7% • Significantly more frequent than in controls	• Prevalence of altered timing, 30.1% (PE, 22.6%; DE, 7.5%) • PE significantly less frequent vs. controls • No significant difference in DE vs. controls	Not assessed	Not assessed	Not assessed
Ferlin et al. [26]	• Case-control • Observational before-after therapy	Infertility• • Testicular hypotrophy	Validated questionnaire	62/60	• IIEF-EF score 26.3 ± 6.6 • No significant difference vs. controls • After TTh in KS: 26.1 ± 5.4, not significant vs. baseline	• IIEF-SD score, 7.3 ± 1.8 • Significantly lower vs. controls • After TTh in KS: 7.9 ± 1.8, $p < 0.05$ vs. baseline	Not assessed	Not assessed	Not assessed	• IIEF-OF score, 9.3 ± 1.8 • No significant difference vs. controls • After TTh in KS: 9.4 ± 1.6, not significant vs. baseline

(continued)

Table 25.1 (continued)

	Study design	Reason for andrological referral	Assessment of sexual function	KS/ controls (*N*)	Erectile function	Sexual desire	Ejaculatory function	Ejaculate volume	Frequency of intercourse	Orgasmic function
Skakkebæk et al. [27]	• Case-control	• KS from endocrine, genetics, and fertility clinics	• Validated questionnaire	132/313	• IIEF-EF score 27 (1–30)	• IIEF-SD score, 8 (2–10)	• Prevalence of PE, 64.8%	Not assessed	Prevalence of reduction (<4/ month), 66.1%	• IIEF-OF score, 9 (0–10)
		• Controls from general population	• Self-rating		• Significantly lower vs. controls	• No significant difference vs. controls	• No significant difference in PE vs. controls		• No significant difference vs. controls	• Significantly lower vs. controls
							• Prevalence of DE, 43.5%			
							• Significantly higher vs. controls			

RCT randomized clinical trial, *T* testosterone, *KS* Klinefelter's syndrome, *ED* erectile dysfunction, *TTh* testosterone therapy, *PE* premature ejaculation, *DE* delayed ejaculation, *IIEF* International Index on Erectile Function, *EF* erectile function domain, *SD* sexual desire domain, *OF* orgasmic function, *NS* not significant

hormone parameters. Cases and controls did not differ for body mass index (BMI) or comorbidities but KS patients had significantly lower T levels. Accordingly, KS men reported reduced sexual desire more frequently than controls whereas premature ejaculation was less prevalent in men with KS. In a following study, Ferlin et al. [26] compared 62 non-mosaic KS patients, evaluated in an Andrology Unit for infertility or testicular hypotrophy, with 60 age-matched healthy men. As compared with controls, KS men had lower T levels, higher BMI, and a worse glycolipid profile. In addition, they scored less on the IIEF-15 erectile function, sexual desire, and satisfaction domains. The sexual desire and satisfaction scores were positively associated with T levels, whereas the erectile function domain was not. Accordingly, among the 25 men who started T therapy, a significant improvement in sexual desire and sexual satisfaction was obtained whereas a non-significant change was found for the erectile function domain. While confirming the findings of the previous studies, which show that sexual dysfunction in KS men is mainly androgen dependent, this study points out that ED has a more complex pathogenesis and metabolic conditions, as well as psycho-relational problems, may be important factors in KS men. In fact, besides having a worse metabolic profile, as aforementioned, KS patients also reported in almost half of cases a history of difficulties in relationships and sexuality during adolescence and, in 10% of cases, body uneasiness or embarrassment. The latest study [27], which considered sexual function in 132 KS men compared with 313 age-, education-, and living area-matched controls, showed, in the largest population published so far, that KS men have a significantly worse erectile and orgasmic function as well as lower intercourse satisfaction. However, this study was not specifically aimed at evaluating the pathogenesis of sexual function in KS men and hormone and glycolipid levels were not part of the assessments, preventing from drawing conclusions on the impact of genetics, hormones, or metabolic impairments in KS men.

25.6 Conclusions

KS men have several risk factors for sexual dysfunctions, including T deficiency, metabolic disorders, and psycho-behavioral conditions. Based on this rationale, sexual function in KS men would be expected to be extensively studied. Quite surprisingly, this is not the case so far. In fact, only a few studies are available and limited progress has been done during the last decade.

Overall, the available studies suggest that KS patients have sexual symptoms as a consequence of T deficiency. Accordingly, their sexual complaints seem to be similar to any other form of hypogonadism rather than being characteristic of the syndrome. However, it should be noticed that these conclusions derive from a limited number of studies mainly involving men who consulted for infertility that could have some peculiarities that preclude the thorough assessment of sexual function. In addition, most of these studies are retrospective reviews of medical charts, and information on sexuality are drawn from available data in records. This limits the quality of the results because studies were not specifically designed for capturing

sexual conditions. In addition, it is conceivable that sexuality was investigated only in men referring a problem rather than being systematically assessed in all KS subjects. This may create a selection bias and could either overestimate sexual problems in KS or underestimate subtle alterations. In particular, the only available study performed in a context of Sexual Medicine [23]—with a systematic assessment of sexual symptoms—found that the prevalence of KS in a population of men consulting for sexual dysfunction is more than eightfold higher than in general population. This suggests that sexuality is an issue in KS and that its characteristics are worth to be investigated. Studies specifically designed and powered for this outcome are hence advocated.

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Testosterone Treatment in Male Patients with Klinefelter's Syndrome

26

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26.1 Introduction

Klinefelter's syndrome (KS) is considered one of the most frequent causes of male organic hypogonadism with an estimated prevalence of 1:500–1:1000 [1–3]. Despite its first description being reported more than 70 years ago, it still represents a great challenge for the medical community since only a minority of those with KS are correctly diagnosed and treated during their life [1, 2]. Accordingly, the vast majority of

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subjects with KS show a mild phenotype, often difficult to distinguish from the general population [1, 2]. Hypogonadism represents one of the most frequent complications observed, with more than 75% of KS affected during their life [2]. Some evidence has suggested that hypogonadism can play a direct role in some of the other frequently observed KS complications such as reduced bone mineral density (BMD), obesity, metabolic disturbances, and increased cardiovascular (CV) risk [4]. Similar considerations have also been reported for the general population [5–8]. However, it is important to recognize that the timing of the testosterone (T) decline appearance in KS still represents an issue of intense debate. Some authors have pointed out the possibility that testosterone (T) deficiency, in KS, is already present during the gestational period [2]. However, available data are quite conflicting. The ratio between the length of the second and fourth digit has been proposed (2D:4D) as well as the anogenital distance as surrogate markers of a child androgen status during pregnancy [2]. Some authors have indeed documented a lower 2D:4D ratio in KS when compared to controls [9, 10]. However, no information on anogenital distance in KS is available. In addition, no difference between KS and controls has been reported in T levels evaluated in amniotic fluid in a small group of KS fetuses [11]. Similarly, T levels were similar in infants and controls [12]. Puberty represents another crucial step in KS, as delayed puberty is described as a possible feature of the condition [1, 2]. However, it should be recognized that puberty occurs normally in the vast majority of patients with KS contributing to difficulty in the diagnosis of the syndrome [1, 2]. Hence, hypogonadism observed in KS is more frequently diagnosed in adulthood. It has been suggested that in KS the age-dependent decline in T observed in adulthood in the general population occurs earlier although direct comparisons are lacking. Similarly, the real contribution of testosterone replacement therapy (TRT) to several outcomes in KS has not been completely clarified.

The aim of the present chapter is to systematically summarize and review all available evidence related to TRT in KS subjects emphasizing and discussing uncertain areas.

26.2 Methods

An extensive Medline, Embase, and Cochrane search was performed including the following words ("testosterone" [MeSH Terms] OR "testosterone" [All Fields]) AND Klinefelter [All Fields] for the selection of studies evaluating the relationship between T and Klinefelter's syndrome on several outcomes. The search, which accrued data from January 1, 1969, up to December 31, 2019, was restricted to English-language articles and studies of human participants.

26.3 Available Testosterone Preparations

The specific description of the available T preparations is beyond the aim of the present study and revised elsewhere [13–15]. Table 26.1 summarizes the most frequently used and available T preparations. No difference has been reported in the

Table 26.1 Available preparations for hypogonadism treatment

Formulation	Chemical structure	T 1/2	Standard dosage	Advantages	Disadvantages
Testosterone preparations					
Oral					
Testosterone undecanoate	17-α-hydroxyl-ester	4 h	120–240 mg, 2–3 times daily	– Reduction in liver involvement – Oral convenience – Modifiable dosage	– Unpredictable absorption depending on meal fat content – Be taken with meals
Parental					
Testosterone enanthate	17-α-hydroxyl-ester	4–5 days	250 mg, every 2–3 weeks	– Low cost – Short-acting preparation allowing drug withdrawal in case of side-effects	– Fluctuations in circulating T levels – Multiple injections – Relative risk of polycythemia
Testosterone cypionate	17-α-hydroxyl-ester	8 days	200 mg, every 2–3 weeks	– Low cost – Short-acting preparation allowing drug withdrawal in case of side-effects	– Fluctuations in circulating T levels – Multiple injections – Relative risk of polycythemia
Testosterone propionate	17-α-hydroxyl-ester	20 h	100 mg, every 2 days	– Low cost – Very short-acting preparation allowing drug withdrawal in case of side-effects	– Fluctuations in circulating T levels – Multiple injections – Relative risk of polycythemia
Testosterone undecanoate in castor oil	17-α-hydroxyl-ester	34 days	1000 mg, every 10–14 weeks *750 mg, every 10 weeks	– Steady-state testosterone level without fluctuation – Long-lasting – Less frequent administration	– Pain at injection site – Long-acting preparation not allowing rapid drug withdrawal in case of side-effects
Surgical implants	Native testosterone	–	4–6 implants of 200 mg lasting up to 6 months	– Long duration and constant serum testosterone level	– Placement is invasive – Risk of extrusion and site infections
Transdermal					
Testosterone patches	Native testosterone	10 h	50–100 mg/day	– Steady-state testosterone level without fluctuation	– Skin irritation – Daily administration
Testosterone gel, 1–2%	Native testosterone	6 h	50–100 mg/day	– Steady-state testosterone level without fluctuation	– Possible transfer during intimate contact – Daily administration

* For the preparation availabe in the United States of America

use of T products between KS and controls. The choice of the T preparation should be discussed with the patients and the final decision should be based on the clinical situation, T formulation availability, and patient needs and expectations [12].

26.4 Early Treatment

Minipuberty is a known physiological phenomenon characterized by the activation of the hypothalamic-pituitary-gonadal (HPG) axis during the neonatal period. It usually occurs in the first 3–6 months of life in both sexes and it allows for the maturation of the sexual organs, creating the basis for future fertility [16]. In addition, some evidence has suggested that the T increase in this period can influence brain development contributing to cognitive functioning and social behaviors [17, 18]. A normal physiological activation of the pituitary-gonadal axis at 3 months of age has been reported in KS similar to what has been observed in healthy boys [19, 20]. However, T levels are significantly lower in KS than in controls [19, 20]. Some evidence has indicated that the rise in androgens observed during minipuberty can influence gray matter volume and cortical maturation leading to an organizational effect of social and cognitive behaviors [21]. Accordingly, structural imaging studies have documented a decrease in gray and white matter volumes in KS when compared to controls [21]. Based on these findings, some others have tested the possibility that an early T treatment during the minipuberty period can result in better cognitive function in KS. Table 26.2 summarizes the most important randomized controlled trials (RCTs) evaluating the effect of an early T treatment in KS infants. In line with the aforementioned hypothesis, some non-placebo controlled trials (RCTs), all derived from the same groups, have suggested that an early T treatment (T enanthate 25 mg, three times) has a positive impact on specific domains of neurodevelopmental function in boys with KS at 36 and 72 months of age [22–24]. However, the effects on cognitive function were not confirmed in the only available placebo controlled RCT [25] in other studies [2, 26]. Hence, present data cannot confirm the utility of an early T treatment in KS although some positive findings have been documented.

26.5 Puberty Management

The management of a delayed puberty in KS does not differ from the general population. About 15–25% of the adult dose is usually sufficient to achieve early virilization and growth over time, without inducing premature closure of the epiphyses. Then the dosage should be progressively increased according to the clinical response up to the normal adult dosage. Several T preparations for oral, transdermal, and intramuscular administration with different characteristics are available (see Table 26.1). The injections each last for a month to 6 weeks and are generally considered more effective than other preparations. A long-lasting ester (i.e., T enanthate) at 50–75 mg/month is used initially and gradually increased to 100–150 mg/month before finally reaching 250 mg dosage every 2–3 weeks. The most used

Table 26.2 Parameters reported per single trial included comparing treated KS patients with controls on several cognitive parameters

	N	Age	Therapy	Placebo controlled	Visual motor function	Psychosocial function	Cognition
Ross et al. [26]	50	4.1–17.8 years	Mixed	No treatment group	NA	NA	↔
Samango-Sprouse et al. [22]	10 XXXY	12 months	25 mg T enanthate (three times)	No treatment group	NA	NA	↑
Samango-Sprouse et al. [23]	34	12 months	25 mg T enanthate (three times)	No treatment group	NA	NA	↑
Samango-Sprouse et al. [24]	29	12 months	25 mg T enanthate (three times)	No treatment group	NA	NA	↑
Ross et al. [25]	84	4–12 months	Oxandrolone (0.06 mg/kg/day)	+	↑	↑	↔
Skakkebæk et al. [2]	69	8–36.4 years	Mixed	No treatment group	NA	NA	↔

KS Klinefelter's syndrome, *NA* not available
↔ = no modification, ↑ = increase; ↓ = reduction, + = with plcebo group

non-injectable forms of T replacement therapy include transdermal gel preparations (1–2%). If T gel is used, the starting dose is 2.5 mg/daily with a gradual increase. Injectable testosterone undecanoate preparation (1000 mg ampoules for i.m. injection) can be applied but should be preferably used for maintenance therapy [27]. Alternatively, the oral route can be used, but sustained blood levels of T are difficult to achieve, because it first passes through the liver. Moreover, the more active 17 alpha alkylated derivatives of testosterone have a potential risk of hepatotoxicity. The only safe oral testosterone treatment is testosterone undecanoate capsules (40 mg each), which must be initially administered once a day with a meal and gradually titrated upwards to two and then three times a day.

26.6 Adulthood Hypogonadism Management

Several observational studies have evaluated the effect of TRT in patients with KS [28–44]. Conversely, only few RCTs are available [45]. Observational studies present important limitations due to the risk of selection bias due to the non-random assignment of T exposure. Accordingly, physicians often prefer to treat healthier individuals, and healthier individuals more often request treatment for their hypogonadism-related problems. In addition, other limitations rely on the lack of information regarding the level of T before and during TRT, as well as on the limited data regarding the type of T preparation used and the follow-up performed during treatment. However, possible advantages include the longer follow-up and the possibility to include a large series of patients. In the following sections, several TRT outcomes in hypogonadal KS will be analyzed.

26.6.1 Body Composition and Metabolic Parameters

Much evidence has documented that KS is associated with worse body composition (high fat mass and lower lean mass content) as well as with impaired glycol-metabolic profile when compared to healthy controls [2]. Whether or not hypogonadism can contribute to this condition in KS is unknown. Table 26.3 reports the most important studies evaluating the effects of TRT on several body composition and metabolic parameters. Overall, available studies include 502 KS with a mean age of 31.5 years, and mean T levels of 10.4 nmol/L. In the vast majority of cases, TRT had a limited effect on several metabolic and body composition parameters. This was confirmed in a recent meta-analysis of the availabe data [3]. Similar data have been reported in the general population. In fact, the most updated meta-analysis on the role of TRT in placebo controlled RCTs showed a possible improvement in lean mass and fat mass without any modification of body weight. Similarly, no modification in lipid profile was observed, although a possible improvement in fasting glucose and Homeostatic Model Assessment (HOMA) index was detected [46, 47]. Accordingly, current European Academy of Andrology guidelines do not support the use of TRT for body composition and metabolic profile improvement [48].

Table 26.3 Parameters reported per single prospective trial included in the analysis comparing untreated with treated KS patients

	KS number	KS age: (mean) years	KS total testosterone: (mean) nmol/L	Treatment duration (weeks)	BMI	Fat mass	Lean mass	FG	HDL	LDL	TC	TG	WC
Ozata et al. [28]	9		3.16						↔	↔			
Ozata et al. [29]	14	21.4	2.7	208									
Ozata et al. [30]	16	20.8		24	↔								
Shibasaki et al. [31]	11	34	8	48									
Yesilova et al. [32]	32	21.66		156	↑						↔	↔	
Bojesen et al. [33]	35	35	12.7	66	↔	↔		↔	↔	↓			↔
Høst et al. [34]	20	36.4	13.6		↔	↔		↔	↔				↔
Jiang-Feng et al. [35]	39	21.5	4.8		↑			↔	↔	↔	↔	↔	
Condorelli et al. [36]	15	53.5	11	52	↔				↔	↔			↓
Jo et al. [37]	18	35.9	3.1		↔								
Pasquali et al. [38]	48	31	13.53	24	↔	↓	↔	↔					
Selice et al. [39]	56		7.3		↔			↔	↔		↔	↓	↔
Chang et al. [40]	50		10.1		↔	↔	↔		↓				
Ferlin et al. [41]	14	3.15	10.5										
Jorgensen et al. [42]	41	36.1	9.9		↔	↔	↔	↔	↓				
Garolla et al. [43]	31	29.4	11.9	68.2	↔								↑
Granato et al. [44]	40	35.8	5.5		↔				↔	↔			
Host et al. [45]	13	34.88	8.5			↔	↔			↔			

BMI body mass index, *FG* blood fasting glucose, *HDL* high density lipoprotein, *KS* Klinefelter's syndrome, *LDL* low density lipoprotein, *TC* total cholesterol, *TG* triglycerides, *WC* waist circumference
↑ = increase, ↓ = decrease, ↔ = no modification

Table 26.4 Parameters reported per single prospective trial included in the analysis comparing untreated with treated KS patients

	KS number	KS age: (mean) years	KS total testosterone: (mean) nmol/L	LS-BMD	FN-BMD
Choi et al. [49]	20	26.5	5.3	↑	↑
Jo et al. [37]	18	35.9	3.1	↑	↔
Ferlin et al. [41]	14	31.5	10.5	↔	↔

FN-BMD femoral neck bone mineral density, *KS* Klinefelter's syndrome, *LS-BMD* lumbar spine bone mineral density
↔ = no modification, ↑ = increase; ↓ = reduction

26.6.2 Bone Parameters

Only three studies have evaluated the effects of TRT on bone mineral density in KS [37, 41, 49]. Overall, they included 52 patients with a mean age of 31.2 years (Table 26.4). The effect of TRT, as reported in the general population [47, 48, 50], was more pronounced at lumbar level when compared to femoral neck (Table 26.4). In addition, similar to what has been reported for the general population [47, 48, 50], no information related to the effect of TRT on bone fracture risk is available. This was confirmed in a recent meta-analysis of the availabe data [3].

Finally, it should be recognized that KS has been associated with a reduced amount of 25-hydroxy −vitamin D levels when compared to controls [41]. Ferlin et al. [41] have reported that KS subjects treated with calcifediol or T + with versus calcifediol had a significant increase in lumbar BMD when compared to those treated with T alone [41].

26.6.3 Mood and Psychiatric Symptoms

Psychiatric symptoms and psychopathological symptoms are quite frequent in KS. Bruining et al. [51] found the presence of learning disorders (65%), attention-deficit hyperactivity disorder (63%), depressive disorders (24%), psychotic disorders (8%), and schizophrenia (2%) in 51 boys with KS. Some case or case series reports have documented a possible improvement in psychopathological symptoms after TRT in patients with KS [52, 53]. However, no data from RCTs are available. In addition, data on the effects of TRT on mood, and depressive symptoms in particular, are conflicting [47, 48].

26.6.4 Sexual Function

A clear association between sexual function impairment and KS is well documented [54–56]. Although, high cardiovascular risk and worse metabolic profile can play an important role in the pathogenesis of erectile dysfunction (ED) in KS, the hormonal component seems to play the major role. Accordingly, much evidence has documented that T is deeply involved in the regulation of all aspects of

sexual function and, in particular, erectile function and libido [57–59]. In line with these data, available meta-analyses have clearly shown that TRT is effective in restoring sexual desire and erectile function only in hypogonadal men (total $T < 12$ nM; see for review [57–59]). In addition, available data showed that sexual effects were proportional to the increase in T concentration and higher on libido, when compared to ED [57–59]. Data on the effect of TRT in sexual function in KS are scanty. In a first pilot observational study including 13 hypogonadal KS patients, Meikle et al. [60] showed that transdermal T patches improved sexual function, increased libido, and decreased fatigue. Similarly, Ferlin et al. [54] showed that sexual function was normalized after 6 months of TRT in 25 hypogonadal KS patients.

26.7 Conclusion

The role of TRT in aging men without well-documented organic causes of hypogonadism still represents a conflicting topic [61–63]. In particular, the cardiovascular (CV) safety of TRT has been questioned [64, 65]. Hence, the concept of organic in comparison to functional hypogonadism is emerging [61]. The present review of the literature indicates that TRT in men with KS, one of the most frequent causes of organic hypogonadism, resulted in similar outcomes to those observed in patients with non-organic or functional hypogonadism. Larger and longer placebo controlled RCTs are advisable to better confirm the present data mainly derived from observational studies.

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