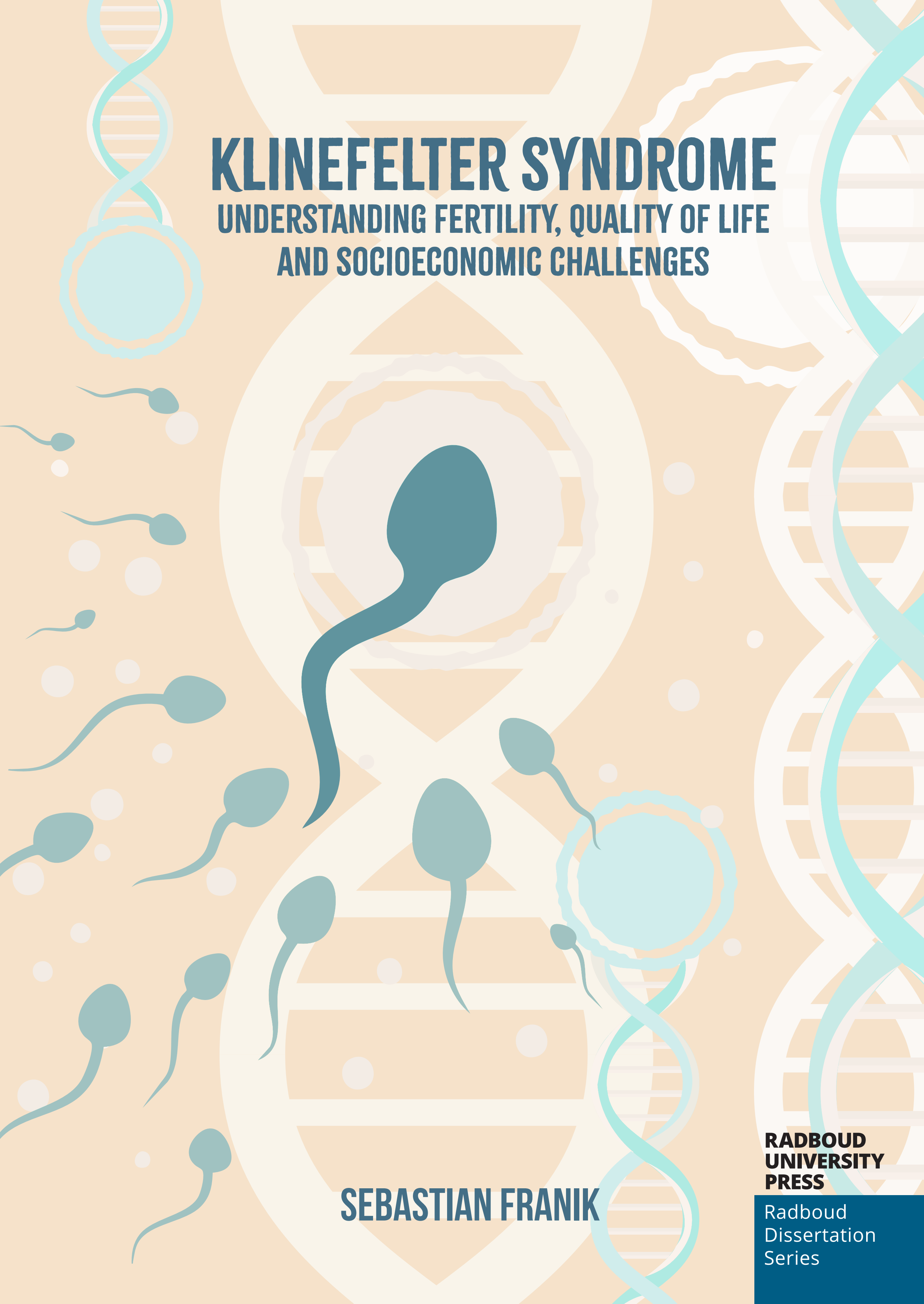

KLINEFELTER SYNDROME

UNDERSTANDING FERTILITY, QUALITY OF LIFE AND SOCIOECONOMIC CHALLENGES

SEBASTIAN FRANIK

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Klinefelter Syndrome

Understanding fertility, quality of life and socioeconomic challenges

Sebastian Franik

The research presented in this thesis was carried out at the department of Obstetrics and Gynaecology, the department of Pediatric Endocrinology, the department of Human Genetics of the Radboud university medical center, Nijmegen, the Netherlands, within the Radboudumc Research Institute for Medical Innovation. This thesis was financially supported by the Radboud university medical center and an unrestricted grant from Merck KGaA.

For reasons of consistency within this thesis, some terms have been standardized throughout the text. As a consequence, the text may differ from the articles that have been published.

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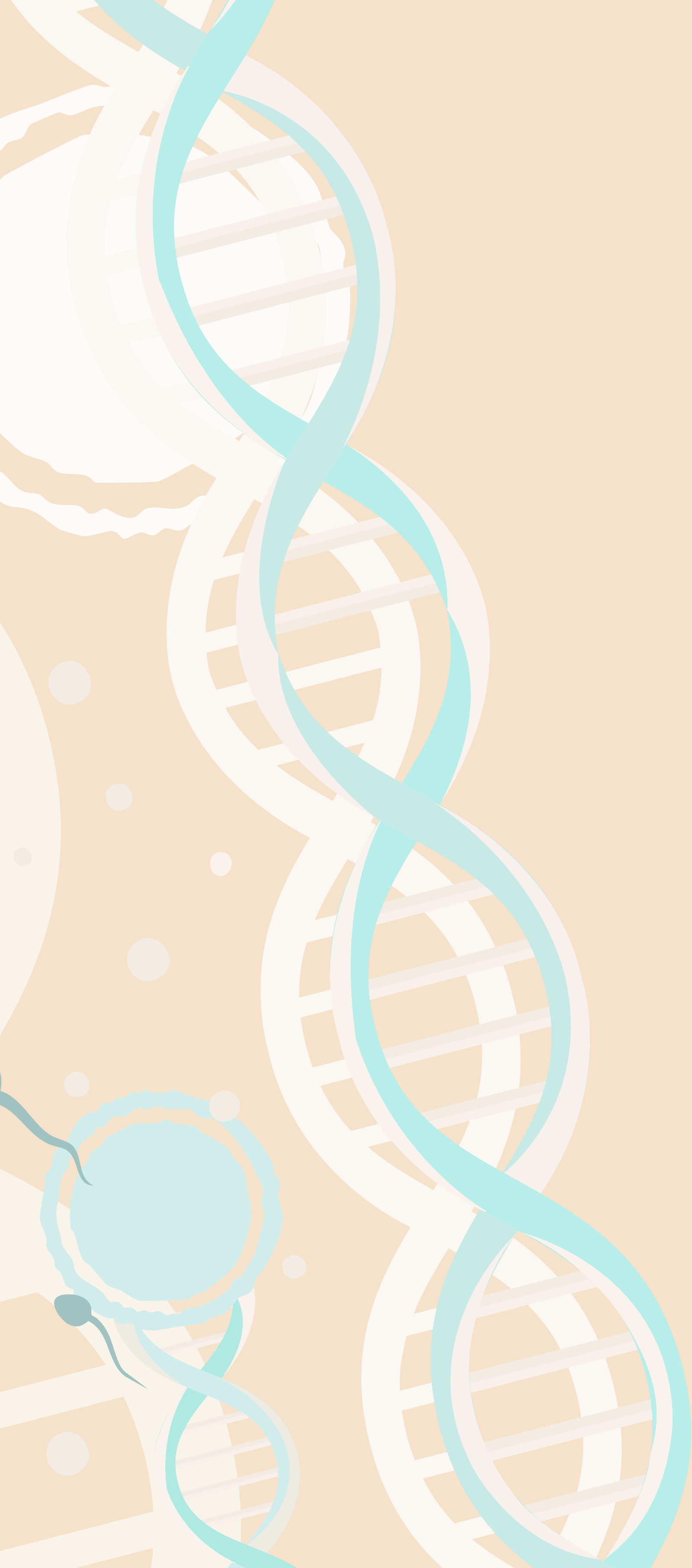

Chapter I

Introduction, aim and outline of this thesis

Introduction

Klinefelter syndrome (KS) was first described in 1942 by E.C. Reifenstein, F Albright and Harry F. Klinefelter who published their essay "Syndrome characterised by gynecomastia, aspermatogenesis without Aleydigism, and increased excretion of follicle-stimulating hormone" in the Journal of clinical Endocrinology in 1942 [1]. The prevalence of KS is approximately 1-2 in 1000 male newborn infants, and rises up to 3-4% in infertile males and up to 12% in males with azoospermia [2]. Up to date, approximately 25% of males with KS receive the diagnosis during lifetime, as we know from registry studies [2,3]. The low rate of detection might be due to a highly variable phenotype and without obvious facial dysmorphology, that make them indistinguishable from boys with a normal karyotype [4]. The presence of mosaicism in about 15% of males with KS, resulting in a 46,XY / 47,XXY karyotype even leads to a higher variability in phenotypic characteristics [5]. Infertility is one of the most serious complications. Other challenging aspects that can occur are learning disabilities, education disparities and discrimination.

Etiology

KS is caused by an additional X chromosome in some cell lines (i.e. mosaicism) or all cells, resulting in an XXY karyotype [6]. About 80-90% of males with KS have a 47,XXY karyotype, while the remaining 20% show a 46,XY/47,XXY mosaicism or higher-grade X aneuploidies, such as 48,XXXY or 49,XXXXY [4,6-8]. Newer research also shows that mosaicis in specific cell lines may be more common in males with KS, for example with a frequency of up to 42% reported in Sertoli cells [9].

The additional X chromosome results from non-disjunction during gametogenesis, a failure of the chromosomes to separate properly during meiosis, the process of cell division that produces sperm or eggs. The exact mechanism of non-disjunction is not fully understood but may be influenced by a combination of genetic and environmental factors, such as maternal or paternal age or exposure to certain chemicals such as environmental toxins, medications, tobacco smoke or alcohol [10-13]. The non-disjunction occurs during oogenesis or spermatogenesis (all cases with non-mosaic KS) or less frequently during early cleavage of the fertilized oocytes (mainly mosaic KS) [14]. The origin of the additional X-chromosome in males with KS is in 50% of maternal origin and 50% of paternal origin [15]. It was postulated that the parental origin of the supernumerary X chromosome and different epigenetic expression is associated with a variance in phenotypic expressions [16,17]. Nevertheless, the connection between the genotype and phenotype in KS remains predominantly uncharted. The tall stature of males with KS can be explained by the over-expression of the SHOX gene (short stature homeobox- gene) located in the pseudoautosomal region 1 (PAR1) on the X chromosome, which is responsible for the regulation of bone growth during fetal development and childhood [18]. Furthermore, the available literature on KS suggests that other treats of the phenotype and the variability in how it is expressed cannot be attributed to a single genetic mechanism. Instead, multiple potential mechanisms, including hypermethylation, variations in mRNA expression, biased X chromosome inactivation, or distinct protein-protein interactions, may operate simultaneously. This complex interplay creates a more intricate perspective on the genomics of KS, with the extra X chromosome causing a comprehensive genomic imbalance that impacts both the epigenome and transcriptome [19].

Clinical characteristics

The clinical characteristics in males with KS vary greatly among affected individuals. KS mosaicism often causes milder clinical symptoms in comparison with 47,XXY karyotype or higher-grade X aneuploidies. The primary characteristic of KS in adults is hypogonadism, which refers to a deficiency in testosterone production. This can result in a variety of physical changes, such as reduced muscle mass and strength and increased body fat [20]. Additionally, KS is associated with small testes, a tall stature and a characteristic body habitus, including long limbs and thin build. But except for small testes, no consistent clinical features or specific abnormalities have been identified. Sexual dysfunction, including reduced libido and difficulties with achieving or maintaining an erection, is also frequently observed in males with KS, which can further impact quality of life [67]. Cognitive and behavioural problems are also common in males with KS [21]. These can include difficulties with language, attention, memory, and executive function, as well as an increased risk for learning disabilities and mental health problems such as anxiety and depression [22]. Fertility is impaired in males with KS, although the degree of infertility can vary greatly among affected individuals. Some males with KS are able to father children with assisted reproductive techniques, while others may be completely infertile [23-25].

In the next sections the most important clinical features of KS are described in more detail.

Testosterone production

The presence of an extra X chromosome in males with KS leads to abnormal development of the testes and consequently diminished gonadal function, resulting in low testosterone production and reduced fertility. The exact mechanisms underlying this process are not fully understood, but several genetic factors have been identified that may contribute. At the onset of puberty, the testes initially grow slightly and then decrease in size. Concurrently, gonadotropin levels increase significantly, reaching the high serum concentrations observed in adults with KS. During puberty, the degeneration of the germline and Sertoli cells accelerates. Additionally, there is extensive fibrosis and hyalinization of the seminiferous tubules, as well as hyperplasia of Leydig cells and fibrosis of the interstitial tissue [21,26]. Nonetheless, localized areas of spermatogenesis may still exist, offering potential for the surgical extraction of viable sperm. This process of hyalinization typically manifests during midpuberty [27]. Upon the onset of puberty in KS patients, levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone are still within the standard range. However, with progression of puberty there is a subsequent increase in FSH and LH levels, accompanied by a decline in testosterone compared to their counterparts without KS [28]. In adult males with KS, a reduction of testosterone, insulin-like factor 3, inhibin B, and anti-Müllerian hormone can be observed. This results in hypergonadotropic hypogonadism with elevated FSH and LH levels, while 17-estradiol and sex hormone-binding globulin (SHBG) levels remain comparable to those of a non-KS control group [68]. Typically, testosterone levels decrease over time to a lower level or even below the normal range [29].

Spermatogenesis

Approximately 90% of the adult males diagnosed with KS experience non-obstructive azoospermia (NOA), defined as the complete lack of sperm in the ejaculate. The remaining 10% show severe oligospermia [30]. NOA is caused by testicular degeneration due to Leydig cell hyperplasia and hyalinization and fibrosis of the seminiferous tubules with consequently impaired production of mature sperm [21]. A possible factor that lead to impaired spermatogenesis in KS is a suboptimal functioning of the SOX13 gene in the sex-determining region of Y (SRY-related HMG-box) on chromosome 1, which has recently been proven to be downregulated in males with KS [31]. Furthermore, the X chromosome contains numerous genes that are involved in male gonadal development and fertility. In males with KS, the presence of an extra X chromosome leads to an imbalance in gene expression between the X chromosomes. Specifically, genes on the additional X chromosome are overexpressed, which can disrupt normal testes development [32]. Genes on the X chromosome that have also been implicated in the development of testicular tissue and male fertility include the SOX3 and SOX9 genes. The overexpression of these genes due to the extra X chromosome can lead to abnormal development of the testes and reduced or absent sperm production [33].

Another relevant aspect in spermatogenesis of KS males is the presence of 46 XY / 47 XXY mosaicism. A small retrospective study reports a larger testicular volume and a higher mean total sperm count for males with mosaic-KS, although it was not reported in which tissue the mosacisim was present [34]. In other studies, a higher presence of mosaicism in testicular tissue was associated with a higher mean total sperm count [35,36]. An isolated testicular mosaicism could explain that sperm is present in testicular tissue in 30-60% of males with mosaic KS, while these are non-mosaic in lymphocyte testing [37]. A novel study has recently shown that loss of the additional X-chromosome in Sertoli cells is crucial for their maturation and the initiation of spermatogenesis in Klinefelter syndrome [38]. This micro-mosaic loss of the X-chromosome allows Sertoli cells to mature and support focal spermatogenesis [38]. Therefore, spermatozoa of men with KS do not have higher risks for aneuploidy compared to non-KS spermatozoa when used for ICSI.

Socioeconomic status and quality of life

KS shows profound socioeconomic implications. These impacts span various aspects of life, including education, employment, income, family life, health, quality of life, and mortality. However, there is limited research on the socioeconomic status of males with KS.

In the realm of education, individuals with KS often face challenges. Many struggle to achieve education, often only managing shorter education. When compared to the general population, fewer men with KS achieve a high level of education [39]. This educational disparity can have long-term effects on their socioeconomic status. Employment is another area where men with KS often find themselves at a disadvantage. They often have lower levels of occupation and are more likely to retire early [39]. This can lead to financial instability and contribute to lower income levels. Satisfaction with current household's income is generally lower among men with KS compared to the general population [40]. This financial strain can further exacerbate the challenges they face. Family life can also be affected. Fewer men with KS live with a partner compared to the general population. Also, fewer become fathers. These factors can lead to feelings of isolation and impact their overall quality of life [39,41]. Health and quality of life are closely intertwined. The presence of health problems and lower scores of subjective general health are related to lower levels of occupation and lower satisfaction with income in men with KS [42]. This highlights the interconnectedness of these socioeconomic factors. Studies have suggested that males with KS may experience a lower quality of life compared to the general population [43,44]. This can be attributed to various factors such as physical and cognitive symptoms, fertility issues, and social stigma associated with the condition. A study found that sexual dysfunction, depression, and anxiety were significant predictors of reduced quality of life in males with KS [45]. Another study showed that low testosterone levels and metabolic syndrome were associated with lower quality of life scores in males with KS [46].

Finally, the poorer socioeconomic status is significantly related to a higher mortality among KS [39]. This sobering fact underscores the seriousness of the socioeconomic challenges faced by individuals with KS.

Given the potential impact of socioeconomic factors on the quality of life and access to healthcare for males with KS, investigating possible associated factors of socioeconomic status would be useful. Identifying such factors could help healthcare providers develop tailored interventions to address socioeconomic disparities in this population. Factors that could be associated with lower socioeconomic status in males with KS may include factors such as educational attainment, employment status, access to healthcare, and quality of care.

Diagnosis

Males with KS are often asymptomatic, have mild symptoms that can be overlooked or have symptoms which are not directly associated to KS such as learning or behavioural disabillties. Therefore males with KS are often undiagnosed. A Danish registry study looking at all prenatal karyotypes from 1970 till 2000 has shown that only 25% of adult males with KS have been diagnosed as such, with less than 10% of them being diagnosed before puberty [2]. Up to date, the number of undiagnosed males with KS has not changed [3]. Most often, the diagnosis KS is suspected in adulthood after a diagnostic work up of infertility. This may change in the future with the availability of non-invasive prenatal chromosome tests during pregnancy (NIPT). The diagnosis of KS is typically made by chromosomal analysis [21]. Chromosome analysis, also known as karyotyping, is a laboratory test used to study the number and structure of an individual's chromosomes. During chromosome analysis, or karyotyping, a sample of cells is taken from the individual and the chromosomes are visualized using a microscope after being stained with a special dye. The chromosomes are then arranged in a standard format and compared to the normal chromosome pattern to look for any abnormalities [14]. With newer high-throughput DNA sequencing technologies such as Next-Generation-Sequencing (NGS), simultaneous sequencing of millions of DNA fragments is possible. For standard karyotyping, is most commonly evaluated in peripheral blood samples, as this is the standard tissue for diagnostic testing. However, NGS offers the flexibility to analyse DNA from various tissues other than peripheral blood, such as skin, saliva or testes, which can be particularly valuable in mosaic KS cases. In KS mosaic cases, the percentage of 47,XXY cells can vary across tissues, with peripheral blood typically showing levels ranging from 10% to 40%, while skin and testicular tissue may exhibit higher mosaicism levels, often between 30% to 70% and exceeding 70% to 80%, respectively [9]. Analyzing DNA from multiple tissues, including skin and saliva, can enhance the sensitivity and accuracy of detecting mosaicism.

After the confirmation of a KS diagnosis, a series of subsequent diagnostic evaluations are typically recommended [21].

For children and adolescents, the most important parameters to be tested are a physical examination including testis and mammary gland evaluation, growth, body proportion and weight. Furthermore, a cognitive evaluation to monitor speech development, learning abilities and psychosocial problems is recommended. It is also important to test vitamin D, calcium, LH and testosterone. During infancy or childhood, a one month topical testosterone therapy is only recommended for cases with a micropenis, while during adolescence regular testosterone therapy is generally recommended in presence of hypogonadism. More details are shown in a current international guideline [21].

For adults, the most important diagnostics are annually physical examinations including testis and mammary gland evaluations, an annually metabolic and hormonal profile, mainly testosterone, LH, vitamin D and calcium determination [21]. Another very important point of consideration is adequate fertility counselling regarding the chances of finding sperm in ejaculate or in the testicular biopsy and the possibility of fertility preservation using cryopreservation. If spermatozoa are present in the ejaculate, it is advised to cryopreserve a semen sample as soon as possible and not only at the time of active childwish, as these males can become azoospermic later in life [47]. In cases where there is an absence of sperm in the ejaculate (azoospermia), a subsequent fertility workup may indicate testicular sperm retrieval (see 1.4.2).

Finally, it is important to evaluate potential learning disabilities, speech impairments, and social disadvantages that can affect individuals with KS [48]. This awareness can facilitate early intervention, which may include specific training or psychological intervention. The type and extent of support provided should be tailored to meet the individual's specific needs. More details are shown in a current guideline (European Academy of Andrology Guidelines on Klinefelter Syndrome [21]).

Treatment: Testosterone deficiency

Low Testosterone levels are a common complication of males with KS, affecting more than 75% of individuals during their lifetime [49]. Therefore, testosterone replacement therapy (TRT) is an important part of the treatment of adult males with KS. Testosterone can be used in various formulations including injections, skin patches, or gels [49]. Testosterone has a positive effect on libido, strength, body hair growth, energy, and mental function such as attention [49]. TRT has also been associated with improvements in body composition and bone mineral density (BMD) at spinal levels [50]. However, it may not ameliorate lipid and glycemic profiles. Despite these benefits, TRT-treated KS subjects still present worse metabolic parameters when compared to age-matched controls [50]. It is important to note that the outcomes of TRT in KS regarding BMD, body composition, and glyco-metabolic control are similar to those observed in males with other forms of hypogonadism.

While most children with KS have a normal start of pubertal development, clinical hypogonadism is also a reason to start with testosterone therapy [51]. The current guideline "recommends testosterone supplementation in case of delayed puberty and/or symptoms of hypogonadism associated with low-normal testosterone and supra-normal LH serum concentrations (LH> 2 SD according to age-related references), after fertility issues have been addressed" [21].

In conclusion, testosterone replacement therapy has been shown to improve many complains associated with KS and improve general health and quality of life, but not spermatogenesis [22].

Treatment: Fertility preservation and assisted reproduction

Given the fact that most males with KS have azoospermia, treatment for subfertility is often required. After the diagnostic workup described earlier, sperm collection is necessary for further treatment of subfertility. Sperm collection includes various non-invasive and invasive techniques. A non-invasive method of male fertility preservation is the collection of spermatozoa from the ejaculate. This is successful in only about 8% of males with KS as only a low percentage have sufficient sperm in their ejaculates to cryopreserve [4]. In case of azoospermia, more invasive methods of sperm collection such as testicular sperm extraction (TESE) are needed [52,53]. TESE is a surgical procedure that is used to retrieve sperm directly from the testes [54]. TESE can be performed under local or general anesthesia. The procedure involves removing a small tissue sample from the testicle to retrieve sperm for use in fertility treatments. There are several different techniques for TESE, including open TESE, multiple sampling and micro-TESE. The approach for conducting an open testicular biopsy can differ. In addition to single biopsies of varying weights (250 mg and 750 mg), the use of multiple smaller biopsies (50 mg each) has been suggested to enhance the success rate of retrieving viable sperm. To consider, with larger biopsies, functional loss of the remaining testicular tissue should to be avoided. Micro-TESE is the most common technique used today for KS as only a small tissue sample from the testicle is removed using a microscope to visualize the testicular tissue. Opaque, dilated tubules are excised from the testis, as they are more likely to contain focal spermatogenesis that is typical for KS NOA [55]. (m)TESE is a highly specialized procedure that requires a thorough understanding of male reproductive anatomy and a high level of surgical skill as these testis have a very small volume. The procedure has a relatively low risk of complication, but as with any surgical procedure, there is a small risk of bleeding, infection, or damage to the surrounding tissue. The testicular trauma of microTESE is lager than conventional TESE but because bipolar, microscopic guided cautary is used, the risk of post operative hemorrhage is considered to be lower, compared to conventional TESE. A recent meta-analysis has shown that there was no difference in sperm retrieval between conservational TESE (cTESE) and micro-TESE (mTESE) in men with Klinefelter syndrome [61].

The sperm retrieved can be stored by cryopreservation, a method where sperm samples are stored in liquid nitrogen at -197°C. The sperm samples can be stored for a variable amount of time, from days to many years [54]. The collected sperm can be subsequently used for further procedures, most commonly intra-cytoplasmatic sperm injection (ICSI) as part of in vitro ferilization (IVF) [56]. ICSI involves the injection of a single sperm directly into the cytoplasm of an oocyte for fertilization in cases with poor sperm quality such as, poor sperm motility, or sperm with abnormal shape and in cases of low sperm count [57]. This technique was first introduced in the 1990s and has since become one of the most widely used procedures in IVF. In 1992, a first study exploring TESE and ICSI in nine males with KS has been published [58]. The IVF-ICSI procedure involves the retrieval of oocytes from the female partner and the selection of a single sperm for injection. The sperm is typically selected based on its morphological characteristics and is carefully examined under a microscope to ensure that it is of good quality. The ICSI procedure is performed under a high-magnification microscope. The fertilized oocyte (after first division, this is called an embryo) is then cultured in a laboratory for 3-5 days, after which it is transferred into the uterus to establish a pregnancy. ICSI has a high success rate and has been shown to improve pregnancy rates in couples facing male factor infertility [54]. However, it is important to keep in mind that there are some risks associated with ICSI, including a slightly increased risk of birth defects and chromosomal abnormalities which are not directly correlated to condition of KS by the paternal line [59]. A recent study has concluded that the paternal genetic risk of sex chromosome abnormalities in the offspring of KS patients is extremely low [60].

The success rate of fertility preservation or treatment with assisted reproduction in men with KS can vary widely, as sperm quality is poor and mostly only a very few sperm is available for treatment. In some cases, no sperm can be retrieved even after TESE as approximately 50% present Sertoli cell only [20]. In these cases, other forms of assisted reproduction, such as using donor sperm, may be necessary [53]. With the availability of TESE in combination with ICSI, spermatozoa can be found in about 50% of males with KS. Unfortunately, live birth rates following ICSI are lower and approximately 16% of the men diagnosed with KS can father their own biological children [20,61].

Organisation of healthcare in KS

Given the challenges of males with KS, healthcare providers need to understand their unique needs and tailor the approach accordingly. Understanding the factors influencing patient satisfaction with care can help healthcare providers tailor their approach to better meet the needs of these patients. Research on patient satisfaction with provided healthcare among men with KS is limited. However, there are some studies that have investigated this issue.

One study conducted in Australia surveyed 144 males with KS to evaluate their satisfaction with medical care [62]. The results showed that overall, males with KS were satisfied with their medical care. However, some males reported a feeling that their concerns were not taken seriously by healthcare providers. Additionally, some males reported feeling uncomfortable discussing issues related to their reproductive health with their healthcare providers. The study concluded that healthcare providers need to be more aware of the unique needs and concerns of males with KS to improve the quality of care they receive [62].

Another study conducted in the Netherlands evaluated 123 males with KS to evaluate their satisfaction with healthcare and their perceived health status [64]. The results showed that the majority of males were satisfied with their healthcare and felt that their healthcare providers were well informed about KS. However, some males reported that their healthcare providers did not fully understand their needs or concerns. Additionally, males who reported more health problems or symptoms related to KS reported lower levels of satisfaction with healthcare. The study concluded that healthcare providers should be aware of the impact of health problems on satisfaction with healthcare in males with KS [64].

Aims and outline of this thesis

Overall, this thesis aims to add knowledge on various clinical aspects of KS, including its impact on fertility, healthcare provision, quality of life, and socioeconomic status. By providing additional background information and justifications for each aim, this thesis will hopefully contribute to a better understanding of this complex condition and ultimately improve long term outcomes for males with KS.

Chapter II: Literature Review - Fertility

In this chapter, we will give an extensive overview about fertility, the major health related problem in KS. KS is characterized by impaired testicular function, leading to various degrees of infertility. Spermatogenesis in KS is characterized by a decrease in the number of germ cells, increased apoptosis of germ cell lines, and an altered ratio of cells in the germline [65]. Sperm recovery techniques, such as TESE and micro-TESE, have been developed to retrieve viable sperm from males with KS, but the success rates are variable and the quality of retrieved sperm is generally poor. Assisted reproductive techniques, such as ICSI, can be used to overcome the poor quality of retrieved sperm, but raise concerns regarding the potential transmission of genetic abnormalities to offspring [66]. Further research is needed to improve the success rates. To give an update of the current scientific knowledge, a systematic literature review was conducted in Chapter 2, answering the following questions:

- What is known in the literature about spermatogenesis and testicular function in KS?
- What are the success rates regarding sperm retrieval in adolescents and adults with KS?

Chapter III: Spermatogenesis

One of the most common features of KS is infertility, which is primarily due to impaired spermatogenesis. The mechanism of impaired spermatogenesis in KS is still not fully understood, but several factors have been identified that contribute to impaired spermatogenesis.

The mechanisms behind impaired spermatogenesis in KS are multifactorial, involving an alternating expression of SHOX genes, disruption of the hypothalamic-pituitary-gonadal axis, abnormalities in the structure and function of the testes, epigenetic modifications, and oxidative stress. The origin of the additional X-chromosome is equally divided between father and mother. Therefore, in Chapter 3 the following research question were investigated:

- Does the inheritance pattern of an additional maternal or paternal X-chromosome predict the presence of any level of spermatogenesis and/or successful sperm retrieval after testicular sperm extraction (TESE) in KS men?

Chapter IV: Evaluation of Healthcare

Studies on the evaluation of health care suggest that males with KS generally report satisfaction with healthcare, but there is room for improvement in addressing their unique needs and concerns. Therefore, the aim of Chapter IV was to evaluate the current health status and co-morbidities of males with KS and assessing their personal satisfaction with the healthcare they have received. Answers will provide valuable insight into the healthcare needs of males with KS and can inform the development of more effective treatment and support strategies

The following research questions guided this investigation:

- How satisfied are males with KS with the healthcare they have received?
- Does the current health status and co-morbidities correlate with the patient´s satisfaction with care?
- What factors contribute to satisfaction with care in males with KS?

Chapter V: Quality of Life

Factors that may influence the quality of life in males with KS include access to healthcare, quality of care, and socioeconomic status. Identifying and addressing these factors can help improve the quality of life for males with KS and reduce the impact of the condition on their overall well-being. Therefore, aims of this study presented in Chapter 5 are:

- To assess the subjective quality of life in males with KS in Europe.
- To identify more possible associated factors on quality of life in males with KS in Europe.

Chapter VI: Socioeconomic Status

By investigating factors such as educational attainment, employment status, access to healthcare and quality of healthcare, we can gain a better understanding of the social and economic determinants of health in males with KS and work towards reducing disparities in this population. Therefore, aims of the study presented in Chapter 6 are:

- To compare the socioeconomic status (SES) in males with KS to a European social surveys (ESS) reference population.
- To investigate possible associated factors of socioeconomic status of males with KS.

Short summary of the aim of this thesis

Overall, this thesis aims to investigate various clinical aspects of KS, from fertility to healthcare provision, quality of life, and socioeconomic status. By providing additional background information and justifications for each finding, this thesis will hopefully contribute to a better understanding of this complex condition and ultimately improve healthcare outcomes for males with KS.

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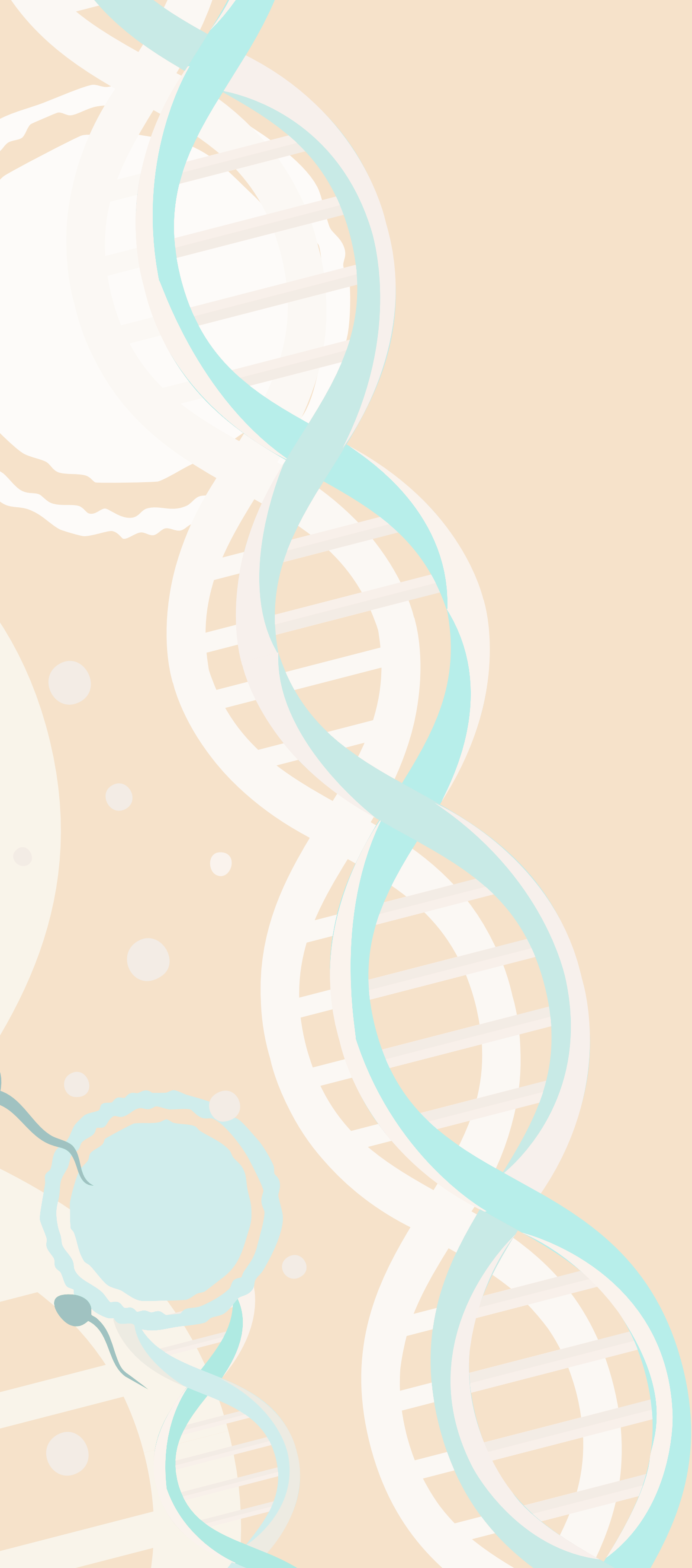

Chapter II

Fertility

Klinefelter syndrome and fertility: sperm preservation should not be offered to children with Klinefelter syndrome

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Abstract

Study Question: Should fertility preservation be offered to children with Klinefelter syndrome (KS)?

Summary Answer: Current evidence shows that fertility preservation should not be offered to adolescents with KS younger than 16 years because of lower retrieval rates for germ cells by testicular sperm extraction (TESE) compared to retrieval rates for adolescents and adults between 16-30 years.

What is known already: KS, the most common chromosomal disorder in men leading to non-obstructive azoospermia, is caused by the presence of at least one additional X chromosome. The onset of puberty in adolescents with KS leads to progressive degeneration of the testicular environment. The impact of the subsequent tissue degeneration on fertility potential of patients with KS is unknown, but in previous literature it has been suggested that fertility preservation should be started in adolescents as early as possible. However, spermatozoa can be found by TESE in about 50% of adults with KS despite severe testicular degeneration. This review discusses the current evidence for fertility preservation in children and adolescents and possible prognostic markers for fertility treatment in KS.

Study design, size, duration: An extensive literature search was conducted, searching Pubmed, Embase, Cinahl and Web of Science from origin until April 2016 for “Klinefelter syndrome” and “fertility” and various synonyms. Titles and abstracts have been scanned manually by the authors for eligibility.

Participants/materials, setting, methods: 76 Studies have been found to be eligible for inclusion in this Review. Information from the papers were extracted separately from SF and YH.

Main results and the role of chance: Various studies have shown that pre-pubertal children with KS already have a reduced number of germ cells despite a normal hormonal profile during childhood. The presence of spermatozoa in the ejaculate of adolescents with KS is extremely rare. Using TESE, the retrieval rates of spermatozoa for adolescents younger than 16 years old are much lower (0-20%) compared to that for adolescents and young adults between 16 and 30 years old (40-70%). Although spermatogonia can be found by TESE in about half of the peri-pubertal adolescents, there are currently no clinically functional techniques for their future use. Children and adolescents need to be informed that early fertility preservation before the age of 16 cannot guarantee fertility later in life and may even reduce the chances for offspring by removing functional immature germ cells which may possibly develop into spermatozoa after puberty. Furthermore, except for the age of patients with KS, there are no identified factors that can reliably be used as a predictive marker for fertility preservation.

Limitations, reasons for caution: Most of the evidence presented in this review is based on studies including a small number of adolescents with KS. Therefore, the studies may have been underpowered to detect clinically significant differences for their various outcomes, especially for potential predictive factors for fertility preservation, such as hormone levels. Furthermore, the population of patients with KS diagnosed during childhood might be different from the adult population with KS where the diagnosis is based on infertility. Results based on comparisons between the two groups must be interpreted with caution.

Wider implications of the findings: Despite the limitations, this review summarizes the current evidence for managing fertility preservation in patients with KS to provide optimal health care.

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Keywords: Klinefelter syndrome; fertility preservation, sperm retrieval;
TESE; adolescent.

Introduction

Klinefelter syndrome (KS) was first described in 1942 by Harry F. Klinefelter and has a prevalence of about 2 in 1000 males [1,2]. About 90% of the cases are due to the 47,XXY karyotype, while the remaining 10% show a 46,XY/47,XXY mosaicism or higher-grade X aneuploidies [3-6]. The phenotype of KS varies greatly: KS mosaicism often causes fewer clinical symptoms in comparison with 47,XXY karyotype or higher-grade X aneuploidies. Typical symptoms include: 1) gynaecomastia and a low sexual drive due to testosterone deficiency; 2) small testes and infertility due to Sertoli cell dysfunction; and 3) psychosocial problems such as concentration problems and learning difficulties [7,8]. Because of the variable phenotype, KS is often not diagnosed. Only 25% of the adult men with KS have been diagnosed as such, with less than 10% of them being diagnosed before puberty [2]. Most often, the diagnosis KS is suspected in adulthood after a diagnostic work up of infertility. Approximately 90% of the adult men diagnosed with KS suffer from non-obstructive azoospermia (NOA) and about 10% from subfertility due to oligospermia [9]. With the availability of assisted reproductive technology (ART) such as testicular sperm extraction (TESE) in combination with intracytoplasmic sperm injection (ICSI), nowadays about half of the men diagnosed with KS can father their own biological children [10]. To date, no markers or clinical parameters have been found to predict spermatogenesis in males with KS.

In this review, we discuss the current literature on fertility preservation and possible prognostic markers for fertility treatment in KS.

Spermatogenesis and testicular function in Klinefelter syndrome

Many studies have investigated the hormonal profile and histological aspects of testis tissue of KS at all ages (Table 1; also compare Davis et al. (2015) [11]. The degeneration of germ cells in KS might be caused by a negative effect of the extra X-chromosome during chromosome segregation by aberrant sexual chromosome silencing or an adverse influence of the supporting somatic cells on germ cells [12]. It remains unclear whether or not germ cell development is normal in early embryonic development. Murken et al. (1974) found that degeneration of germ cells starts in fetal life [13]; another study by Coerdt et al. (1985) showed that there is a significantly reduced number of germ cells in testicular biopsies from 47,XXY midterm fetuses, although the number and density of testicular tubules and mesenchymal structures appear to be normal [13,14]. Two further studies describe normal histology in early fetal testes biopsies [15,16]. In healthy neonates, the pituitary-gonadal axis is strongly activated, producing pubertal or even adult levels of serum follicle stimulating hormone (FSH), luteinizing hormone

(LH), and consequently testosterone and inhibin B until the age of 6 months. This is also called "the mini-puberty" [17]. After the mini-puberty, the hormone levels decrease to pre-pubertal levels until the pituitary gonadotropin axis is reactivated during puberty. In 2004, Lahlou et al. showed that in infants with KS testosterone levels were elevated during the first months of life, indicating the presence of a mini-puberty [18]. However, their testosterone levels were significantly lower when compared to infants with a normal karyotype, indicating a disturbed function of the Leydig cells [18]. In contrast, several papers describe a normal development of Sertoli and Leydig cells but with a reduced number of germ cells during early fetal life [19-21]. During childhood, there are normal prepubertal hormonal levels of testosterone, sex hormone binding globulin (SHBG), FSH, LH, inhibin B, anti-müllerian hormone (AMH), estradiol (E2) but a reduced number of spermatogonia in KS [22-32]. To conclude, the reproductive potential of pre-pubertal children with KS seems to be already compromised, but the precise mechanism remains as yet unknown [11,33,34].

During puberty, major histological changes in the testes occur. Initially, the testes of KS adolescents grow up to a volume of 6 ml due to the proliferation of Sertoli cells and interstitial cells [31]. However, rising serum testosterone levels are subsequently followed by an accelerating decline of germ cells, hyalinization of the tubules, degeneration of Sertoli cells and hyperplasia of Leydig cells [31]. This degeneration of the interstitial stroma is accompanied by a decrease in the testis volume to a prepubertal size of 2-4 ml [35,36]. It is still unclear whether or not the rise in serum or intratesticular testosterone concentrations during puberty is associated with accelerated destruction of the seminiferous tubules during this period [11].

Sperm recovery in ejaculates and TESE in KS

Adolescents

To our knowledge, four studies have been published reporting sperm recovery attempts by ejaculation in adolescents and young adults with KS (Table 2). In three studies, no spermatozoa were found in the ejaculates of 27 participants (in total) aged 10 to 25 years [37-39]. In the fourth study, a severe oligoasthenoteratozoospermia was found in one 17-year-old adolescent, whereas in the remaining 27 participants aged 13-19 years, no spermatozoa were found [40]. Information about the effectiveness of TESE in adolescents with KS is more abundant (Table 2). The first case report of a successful TESE in a 15 year old boy with non-mosaic KS was published in 2001 [41]. A subsequent study investigated the effectiveness of TESE in 14 boys with non-mosaic KS aged 10-14 years. They found no spermatozoa in the study group, but spermatogonia were found in 50% of the children [31]. Another study confirmed these findings by reporting the absence of spermatozoa in all seven boys with non-mosaic KS aged 13-16 years. Again, spermatogonia were found in 4 out of 7 boys [38]. Rives et al. reported the presence of spermatozoa by TESE in 1 of 5 boys with non-mosaic KS aged 15 to 16 years, and the presence of elongated spermatids in another one [42]. Van Saen et al. reported retrieval rates of spermatogonia of 72% (5/7) in adolescents with KS aged 13-16 years. However, the overall number of spermatogonia in the boys with KS was significantly reduced compared to the number of spermatogonia found in normal adolescent boys. No spermatozoa were found in the adolescents boys with KS [28]. Another study by Rohayem et al. investigated the retrieval of spermatozoa by microscopically assisted TESE (mTESE) in a study population of 50 adolescents aged 13 to 19 years [40]. They reported a spermatozoa retrieval rate of 38% (19/50). However, in the subgroup of adolescents aged 13-14 years, the spermatozoa retrieval rate was 10% (1/10), whereas in the subgroup of adolescents aged 15-19 years the spermatozoa retrieval rate was much higher with 45% (18/40) [40]. Another recent study investigated the spermatozoa retrieval rate by mTESE in 10 adolescents and young men with KS aged 12-25 years and found spermatozoa retrieval rates of 50% (5/10) [39]. A recent study by Plotton et al. investigated the effectiveness of TESE in 21 males with non-mosaic KS aged 15 to 20 years; spermatozoa retrieval rates of 57% (12/21) were achieved [43]. Even higher sperm retrieval rates of 70% were reported in a small study investigating the effectiveness of pre-treatment with testosterone and the aromatase inhibitor letrozole in 10 adolescents with non-mosaic KS aged 14-22 years [44].

To summarize, spermatozoa are extremely rare in ejaculates of adolescents and young adults with KS. The spermatozoa retrieval rate by TESE in adolescents with KS varies between 0% and 70% in different small studies, mostly depending on the age of the participants (Table 2). The presence of spermatogonia in testicular biopsies in adolescents with KS has been reported to be about 50%.

Adults

Four case reports have shown the presence of spermatozoa in the ejaculate of adults with non-mosaic KS and their ability to father spontaneously their genetically own child by reporting live birth [45-48]. Furthermore, five larger studies reported uniformly successful ejaculation of spermatozoa in 7.4-8.4% of their study population of 351 men (in total) with KS [4,37,49-51]. Two of those men were diagnosed as having a mosaic pattern; the other 349 had non-mosaic KS confirmed by lymphocyte karyotyping.

The underlying mechanism leading to the ejaculation of sperm in about 8% of apparently non-mosaic KS has not yet been revealed. One possibility could be that a blood mosaicism has simply been overlooked, because most frequently only 20-30 lymphocytes are tested. Another possible explanation could be a higher rate of hidden mosaicism in testicular tissue compared to lymphocytes, as has been shown in a study investigating 5 men with KS where mucosal cells and testicular tissue had a much higher rate of mosaicism compared to lymphocytes [52].

The effectiveness of TESE in adults with KS has been carefully reviewed in 2013 [53]. A total of 22 studies investigating TESE or mTESE in 741 adults with KS, reported 374 successful spermatozoa retrievals by (m)TESE procedures, which is an overall sperm retrieval rate of 50% [53]. However, for adults where no spermatozoa have been found by TESE, there is still an 18% chance of finding spermatogonia [29]. The live birth rate per embryo transfer for an ICSI procedure for KS after successful sperm retrieval by TESE was shown to be similar to the success rate reported for other causes of non-obstructive azoospermia: 28% of 33 patients compared to 26% of 113 controls [54].

In conclusion, about 8% of adult men with apparently non-mosaic KS are able to ejaculate spermatozoa (Table 3), and sperm retrieval rates by (m)TESE are reported to be about 50%.

Experimental approaches for sperm recovery by using immature germ cells in children and adolescents

During the past decade, important achievements have been made regarding fertility preservation. Different approaches for preserving fertility with immature germ cells have been studied [55,56]. Cryopreservation or vitrification of either testicular tissue or spermatogonial stem cell (SSC) suspension is currently the method of choice for malignancy, although it is still experimental [57-60]. Pre-clinical studies harvesting immature germ cells in primates have shown the capability of generating sperm by transplantation of the previously extracted tissue [61]. However, for KS boys there are some concerns regarding the feasibility of this technique [38]. It is unknown whether the XXY karyotype originally affects somatic cells or germ cells. If the karyotype affects the somatic cells, a re-transplantation of harvested germ cells might be unsuitable because of the subsequent hyalinization of testicular tissue. This needs to be investigated before approaches such as transplanting testicular tissue or SSCs for maturation could be considered for fertility preservation of Klinefelter boys. Another approach called in-vitro maturation is still experimental and not yet proven to be safe in humans. Despite great achievements in animal studies, no complete in-vitro spermatogenesis has yet been accomplished in humans [56,62]. A study investigating in-vitro maturation of human germ cells from non-obstructive azoospermic patients showed proliferation of spermatogonia and the differentiation of elongated spermatids. But the spermatids had a low fertilization potential and almost all derived blastocysts showed severe chromosomal aberrations [63].

To summarize, fertility preserving approaches for KS children using spermatogonial stem cells remain highly experimental. Unfortunately, it is unlikely that techniques such as tissue grafting, SSC transplantation, xenografts or in-vitro maturation of SSCs will produce viable spermatozoa in KS men within the next few years.

Predicting factors for sperm retrieval

So far, no consensus has been reached about the optimal age for successful sperm retrieval in KS [33,43,50,64-69]. Four studies including a total of 169 participants observed that an age above 34 years may have a negative impact on sperm retrieval [64,65,66,67]. Another four studies including 151 KS men reported no differences regarding successful sperm retrieval by TESE in participants aged between 15 and 33 years old [43,50,68,69]. A study by Plotton et al. compared the effectiveness of TESE in 25 participants aged 16 to 24 with 16 participants aged 25 to 39 years and found similar sperm retrieval rates of 13/25 (52%) and 10/16 (63%), respectively [43]. For adolescents with KS younger than 15 years, current evidence suggests that the retrieval rate of germ cells by (m)TESE is very low compared to adolescents of 15 years and older [31,33,38,40,42]. It is clear that the degeneration of the testicular environment seems to accelerate with the onset of puberty, but there is no evidence yet for a negative influence on spermatogenesis. As Oates et al. (2012) stated, there have been no studies conducted investigating the sperm retrieval from ejaculation or TESE for the same patient at multiple times in subsequent years during sexual development [34]. Although the study with the lowest average age in Table 4 [43] has the highest retrieval rate of spermatozoa (56%), an age-dependent decline of retrieval rates cannot be estimated at present (Table 4).

It has to be noted that the population of KS patients diagnosed during childhood may be different from the population with KS diagnosed at adulthood. Children with KS are usually diagnosed due to behavioral and intellectual problems, while adults are usually diagnosed due to infertility without other symptoms [70,71]. Therefore caution is required when comparing results obtained in boys with those obtained in adults.

The impaired spermatogenesis in half of KS patients could also be caused by an intrinsic problem of the germ cells, possibly linked to (epi)genetics of the surplus X-chromosome instead of being a result of the hyalinization and fibrosis of the testicular environment. Support for this theory would be the stable sperm retrieval rate of around 50% among KS men and the failure of progressive tissue degeneration to be of prognostic value for spermatogenesis [53]. However, in various small studies, the inactivation pattern of the surplus X-chromosome was also not shown to be of predictive significance for phenotypic characteristics [30]. Other factors such as hormones (serum FSH, LH, free and total testosterone, E2, inhibin B, SHBG, prolactin), testicular volume and testicular histology have also not shown any predictive value for higher sperm retrieval rates [28,38,50,64,69,72-74]. Only Rohayem et al. (2015) described a combination of total serum testosterone above 7.5nmol/L and LH levels below 17.5U/L to result in higher retrieval rates of spermatozoa by mTESE in both children and adults with KS [40]. Another large study has identified higher serum testosterone levels and lower levels of LH and FSH as positive predictive markers in men with azoospermia in general, but the predictive value of serum testosterone levels in KS remains unclear [75].

Treatment with testosterone has previously been reported to be a negative influence on future fertility treatment of KS, based on a sperm retrieval rate of 20% within a small study population of five adults with KS [76]. In contrast, Plotton et al. (2015) found no negative influence of testosterone treatment on spermatogenesis in 41 adolescents and adults [43]. Overall, they reported spermatozoa retrieval by TESE in 9/17 (52.9%) men with KS who had previously been treated with testosterone, and a positive TESE in 14/24 (59.1%) men with KS who never had been treated with testosterone [43]. Another study including 10 adolescents and young men with KS aged 14-22 years who received topical testosterone replacement therapy and an aromatase inhibitor, found spermatozoa by TESE in 7 of 10 participants (70%) [44]. Continuing topical testosterone replacement therapy during fertility preservation, as chosen by Mehta et al. (2013), or stopping the testosterone treatment nine months before performing TESE, as arbitrarily chosen by Plotton et al., (2015), has yielded good results [43,44]. Therefore, testosterone treatment is not likely to have a permanent negative impact on fertility treatment, but larger studies are necessary to confirm these findings. Current evidence does not justify postponing clinically-indicated androgen treatment in adolescent boys with KS for fear of reducing their future fertility and TESE prospects.

Clinical approach to achieve fertility in KS

Despite the common advice for adolescents with KS to undergo TESE as early as possible as being reported in some literature, we suggest a more expectant approach as previously recommended [33,34]. Based on the studies described in this work, (pre)pubertal TESE cannot be recommended to date. Parents of children with KS need to be clearly informed that (pre)pubertal TESE is highly experimental and that this approach cannot guarantee fertility later in life. Indeed it even bears the risk of harming the fertility of their offspring, by possibly removing SSCs that may have had developed into spermatozoa later in life. Furthermore, the psychological impact of a TESE on a young adolescent, who may already have psychological problems due to abnormal sexual development, should be taken into consideration. Two recent studies show that fertility is not an issue of awareness in adolescent boys with KS, but that their parents and pediatricians appreciate fertility preservation at an early age, despite the experimental character of the intervention [42,77]. The impact of a TESE procedure on the psychological well-being of adolescents with KS has not been investigated and studies are needed.

Conclusion

Current literature shows that spermatozoa can be found by TESE in half of the patients with KS aged 16-30 years old. Therefore, we suggest that a TESE procedure for fertility preservation should not be performed in KS patients younger than 16 years, except for patients in an academic research setting, but it is best performed before the age of thirty, even if these men prefer to postpone fatherhood.

Authors' roles

SF and YH searched the databases, did the data extraction and wrote the first draft of this review. SF also wrote the final draft. KD, DB, WN, DS, HC, LR and KF contributed to the review by helping with the interpretation of the data, revising the article critically and writing the final draft.

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Conflict of Interests

S. Franik, Y. Hoeijmakers, K. D’Hauwers, W.L.M. Nelen, D. Smeets, H.L. Claahsen – van der Grinten and L. Ramos declare to have no conflicts of interests.

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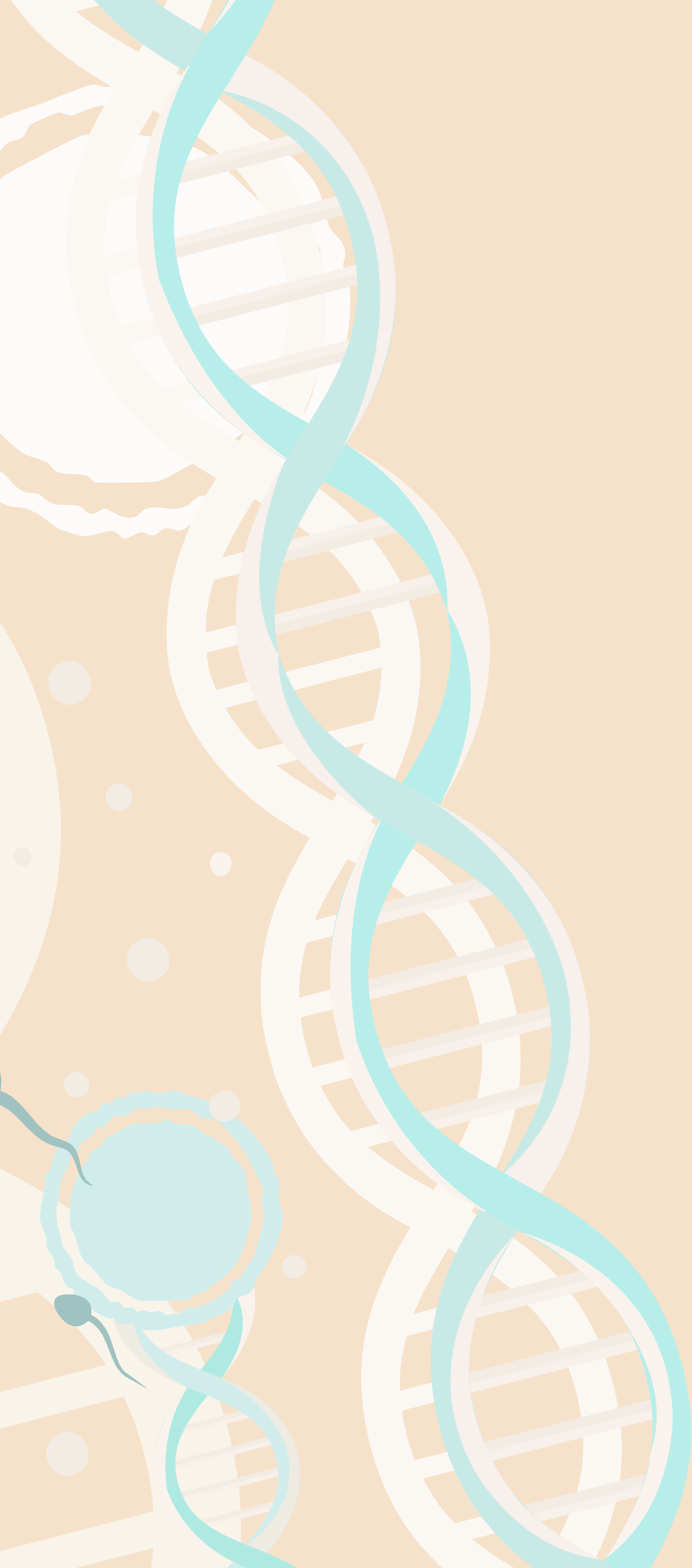

Chapter III

Fertility

Klinefelter syndrome and fertility – Impact of X-chromosomal inheritance on spermatogenesis

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Abstract

With the use of testicular sperm extraction (TESE), spermatozoa can be retrieved in about 30%-50% of men with Klinefelter syndrome (KS). The reason for the absence or presence of spermatozoa in half of the men with KS remains unknown. Therefore, the search for an objective marker for a positive prediction in finding spermatozoa is of significant clinical value to avoid unnecessary testicular biopsies in males with (mostly) low testicular volume and impaired testosterone. The objective of this study was to determine whether paternal or maternal inheritance of the additional X-chromosome can predict the absence or presence of spermatogenesis in men with KS. Men with KS who have had a testicular biopsy for diagnostic fertility workup TESE were eligible for inclusion. Buccal swabs from nine KS patients and parents (trios) were taken to compare X-chromosomal inheritance to determine the parental origin of both X-chromosomes in the males with KS. Spermatozoa were found in TESE biopsies 8 of 35 (23%) patients after performing a unilateral or bilateral TESE. Different levels of spermatogenesis (from the only presence of spermatogonia, up to maturation arrest or hypospermatogenesis) appeared to be present in 19 of 35 (54%) men, meaning that the presence of spermatogenesis not always yields mature spermatozoa. From the nine KS-trios that were genetically analysed for X-chromosomal inheritance origin, no evidence of a correlation between the maternal or paternal origin of the additional X-chromosome and the presence of spermatogenesis was found. In conclusion, the maternal or paternal origin of the additional X-chromosome in men with KS does not predict the presence or absence of spermatogenesis.

Introduction

Klinefelter syndrome (KS) is one of the most prevalent sex chromosomal disorders with an incidence of about 1 to 2 per 1,000 male newborns [1]. It is based on an X-chromosomal polysomy, with X-disomy being the most common type of Klinefelter [2,3]. Interestingly, the diagnosis of KS is often made after a diagnostic workup on infertility in adults, which might suggest that the real incidence might be even higher in the general population [4]. About 90% of men with KS suffer from nonobstructive azoospermia (NOA) and about 10% from subfertility due to severe oligospermia [5]. The development of intracytoplasmic sperm injection (ICSI) in combination with testicular sperm extraction (TESE) makes it possible for men with KS to father of their own biological children. However, only in 30%-50% of men with KS undergoing TESE, spermatozoa can be retrieved from the testis 6. The aetiology for the presence or absence of spermatogenesis in Klinefelter patients is still unclear as no pathophysiological mechanism determining the absence or presence of spermatozoa has yet been revealed. Except for age and hormonal values of LH and testosterone, no prognostic markers or parameters have been found that could predict spermatogenesis in these males [7-8]. However, both parameters are only very weak predictors. Searching the possible mechanisms in predicting spermatogenesis to avoid performing unnecessary biopsies in males with a compromised testicular volume and testosterone production, we hypothesized that the origin of the additional X-chromosome might be a clinical marker for spermatogenesis. Phenotypic differences have already been described in the literature for males carrying an additional paternal X-chromosome [9]. Therefore, it might be of diagnostic value to determine whether the extra X-chromosome is inherited paternally or maternally before starting fertility treatment. As about 10% of the genes located on the X-chromosome are expressed in the testis, these X-chromosomal genes are likely to influence spermatogenesis by over- or under-expression of testicular gene products, which might impair testicular physiology and the production of germ cells [10]. The objective of this study was to explore whether the inheritance of an additional maternal or paternal X-chromosome in Klinefelter men can predict the presence of any level of spermatogenesis and/or successful sperm retrieval after TESE.

Methods

Study participants

A total of 35 adult males with nonmosaic KS (47,XXY) and NOA who underwent a diagnostic TESE procedure at the Radboud University Medical Centre between 2010 and 2016 were eligible for inclusion in the X-chromosomal inheritance study. Before 2010, no TESE could be performed in chromosomal abnormal patients due to a national law. An additional inclusion criterion for the genetic analysis was the ability of at least one biological parent to donate saliva samples for X-inheritance testing. Exclusion criteria for participation in this study were as follows: a language barrier to explain the procedure; no informed consent signed; impossibility to obtain a saliva sample from at least one parent. All patients gave written informed consent to the publication of their case details. Serum testosterone (nmol/L), FSH (U/L), LH (U/L) and inhibin B (U/L) were measured prior to TESE in all participants using enzyme-linked immunosorbent assay (ELISA). A short questionnaire including age, BMI and personal information about smoking, alcohol consumption and drug abuse was filled in by all participants. The questions asked included "Do you currently smoke cigarettes regularly? If yes, how many cigarettes per day?", "How much units of alcohol do you drink per week?" and "Do you use substances such as marihuana or other illegal drugs?". One unit of alcohol was defined as 0.2 L of beer or wine or 2 cl of strong alcohol. A consumption of up to 14 units of alcohol per week was defined as moderate alcohol consumption.

DNA isolation and analysis

A DNA sample was collected from each participant using a sterile cotton swab for a mucosa sample. Subsequently, DNA was isolated from the swabs using QIAamp DNA Mini Kit. The isolated DNA was amplified by QF-PCR using fluorescent-labelled Aneufast™ V3 primerset S1, S2 and MXY. The fluorescent DNA fragments were subsequently analysed (GeneScan) using Applied Biosystems DNA Analyzer 3730. The data from the gene array were analysed using SoftGenetics GeneMarker (IG), a genescan analysis software from the sequence facility (Seqfac). Genotyping and analysis of the markers (AMXY, DXYS267, D21S1414, D21S1446, D21S1442, SRY, X22, DXYS218, HPRT) was performed with the SoftGenetics GeneMarker (IG) software. The results of the analysis were evaluated by three researchers independently.

Testicular sperm extraction, cytology and histology analysis

Testicular biopsies of at least 10 × 5 mm were obtained by our standard procedure and immediately transported to the fertility laboratory [11]. Testicular biopsies were prepared and evaluated for the presence or absence of spermatozoa using wet mount preparation. The presence of at least one spermatozoon was considered a positive sperm retrieval (TESE+). Cytology evaluation by scoring the ratios between spermatozoa, pachytene spermatocytes and Sertoli cells was also performed on the TESE samples, using a technique recently introduced [11]. The cell suspension obtained was prepared on a glass slide, and at least 400 cells were evaluated per slide at a 1000 × magnification. A second observer randomly confirmed the cell counts and spermatogenic patterns for validation. The ratios between the counted Sertoli cells, meiotic (pachytene) spermatocytes and testicular spermatozoa were calculated as a measure for the productivity of the meiotic cells. Furthermore, we carried out a histopathological analysis of the spermatogenesis for each tissue sample collected by TESE and analysed the structural changes within the testicular tissue using Giemsa-stained smears.

Statistical analysis

A descriptive statistical analysis was performed using IBM SPSS statistics version 22. Mean and standard deviation was calculated for continuous variables. Unpaired student t test and Pearson's chi-squared tests were performed to test for goodness of fit and comparison of the baseline characteristics including hormone levels.

Ethical approval

Ethical approval was given by the local ethics committee from the Radboudumc, Nijmegen, The Netherlands.

Results

Nine of the 35 eligible participants took part in the study. The baseline characteristics of the nine included participants are shown in Table 1. No statistically significant difference was observed in the baseline characteristics between the TESE-positive and the TESEnegative participants. A paternal origin of the extra X-chromosome was found in six of nine (67%), and a maternal origin was found in three of nine (33%) participants. From the three TESE-positive participants, a paternal origin of the extra X-chromosome was found in two males (67%) and a maternal origin was found in one male (33%). From the six TESE-negative participants, four (67%) had a paternal origin and two (33%) had a maternal origin of the extra X-chromosome. No evidence was found for an association between the maternal or paternal origin of the extra X-chromosome and the presence of spermatozoa retrieval by TESE or the presence of spermatogenesis ($p = .69$). The descriptions of Xchromosomal inheritance and spermatogenesis are shown in Table 2. The results of our analysis on spermatogenesis were not influenced by the evaluation of a unilateral or bilateral biopsy. All participants without spermatozoa retrieval had a bilateral TESE. While in only three cases spermatozoa was obtained for ICSI, spermatogenesis at different maturation stages was observed in histological or cytological preparations. In five of nine (56%) participants, various stages of spermatogenesis were identified in the histological analysis (varying from the presence of spermatozoa in three patients to the presence of pachytene spermatocytes in another two patients). The results of our cytological analysis, as previously introduced by Hessel et al., showed very low ratios of spermatozoa/pachytene spermatocytes in two of nine (22%) participants (Hessel et al., 2015). Maturation arrest was present in one (11%) participant upon cytological analysis. No evidence for a difference in age between the two subgroups with or without the presence of spermatogenesis was observed (p = .09). Analysis of hormonal levels as measured prior to the TESE did not show any statistical difference between the TESE-positive and TESE-negative subgroups with regard to the mean levels of testosterone (11.5 ± 6.8 vs 8.9 ± 6.8, p = .64), LH (14.6 ± 0.85 vs 19.1 ± 3.2, p = .11) and FSH (26.6 ± 5.1 vs 39.0 ± 17.4, p = .42). In our study population, the thresholds for sperm production previously suggested by Rohayem et al. (2015) (serum testosterone >7.5 nmol/L and serum LH < 17.5 U/L) could only predict spermatozoa retrieval in two cases (67%, p = .06). Inhibin B was under the detection level in all patients who underwent TESE.

TABLE 1 Baseline characteristics of the study participants

	TESE positive (*n* = 3)	**TESE negative (*n* = 6)**	**Statistical significance (unpaired student's *t* test)**
Age in years (mean ± *SD*)	38.7 ± 3.06	33.00 ± 4.65	*p* = .37
Smokers	1/3 (33%)	2/6 (33%)	*p* = 1
Moderate alcohol consumption (up to 14 units of alcohol per week)	2/3 (67%)	5/6 (83%)	*p* = .72
Drug abuse	0/3 (0%)	0/6 (0%)	*p* = 1
BMI (mean ± *SD*)	26.5 ± 3.3	24.1 ± 3.8	*p* = .36
Hormone levels prior to TESE			
Follicle stimulating hormone (U/L)	26.6 ± 5.1	39.0 ± 17.4	*p* = .42
Luteinising hormone (U/L)	14.6 ± 0.8	19.3 ± 3.1	*p* = .08
Testosterone (nmol/L)	11.5 ± 6.9	8.9 ± 6.8	*p* = .66
Inhibin B (U/L)	<10	<10	*p* = 1
X-chromosomal origin			
Paternal	2/3 (67%)	2/6 (33%)	*p* = .69
Maternal	1/3 (33%)	4/6 (67%)	

TESE, testicular sperm extraction.

TABLE 2 Results of X-chromosomal inheritance pattern analysis and testicular sperm extraction (TESE)

Participant	Extra X-chromosomal origin	TESE[a](wet-prep)	Cytology[b]	Histology
P2	Paternal	Positive	Hypo-sperm.	Abnormal testis parenchyma, but some tubuli seminiferi with low levels of spermatogenesis.
P10	Paternal	Negative	SCO	Leydig cell hyperplasia and tubular hyalinisation, no spermatogenesis.
P15	Maternal	Negative	Not performed	Leydig cell hyperplasia, tubular hyalinisation and Sertoli cell only, no spermatogenesis.
P17	Paternal	Positive	SCO	Leydig cell hyperplasia and tubular hyalinisation, but some tubuli with spermatogenesis (<10%)
P21	Maternal	Negative	SCO	Leydig cell hyperplasia and tubular hyalinisation, no spermatogenesis.
P23	Paternal	Negative	MA	Leydig cell hyperplasia, tubular hyalinisation and Sertoli cell only, no spermatogenesis.
P24	Paternal	Negative	SCO	Leydig cell hyperplasia and tubular hyalinisation, but some tubuli with spermatogenesis (<5%)
P28	Paternal	Negative	SCO	Leydig cell hyperplasia and tubular hyalinisation, no spermatogenesis.
P35	Maternal	Positive	Not performed	No histology done, not enough material.

SCO, Sertoli cells only; MA, maturation arrest (presence of pachytene spermatocytes); hypo-sperm, hypospermatogenesis (presence of mature spermatozoa next to pachytene spermatocytes).

[a] Positive sperm retrieval by TESE.

[b] Cytology performed based on ratio of number of Sertolli cells/pachytene spermatocyte/spermatozoa. The outcome is given as: Sertoli cell only (SCO)/ maturation arrest (MA) or hypospermatogenesis (Hypo-sperm).

Discussion

In our study, we investigated whether a paternal or maternal origin of the second X-chromosome was associated with presence or absence of spermatogenesis and the chance of finding spermatozoa either in the ejaculates or testis biopsies of KS males. Unfortunately, the results show that neither maternal nor paternal origin of the extra X-chromosome can predict the presence or absence of spermatogenesis. To our knowledge, this was the first study investigating X-chromosomal origin in combination with the presence of spermatozoa by TESE. The possible impact of maternal or paternal X-chromosomal origin in KS was previously investigated in a prospective study including 14 nonmosaic boys with KS 10-14 years old, that suggested a later onset of puberty in KS with a paternally inherited supernumerary X-chromosome [12]. Another study investigated the inheritance pattern of the extra X-chromosome in 17 men with KS and found a maternal origin in 10 of 17 (59%) participants and a paternal origin in seven of 17 (41%) participants [13]. Furthermore, they found skewed X-chromosomal inactivation (same X-chromosome inactivated in >80% of cells) in three of 14 participants (21%) and a varying degree of X-chromosomal inactivation pattern in the other patients (ranging from 30% to 69%) [13]. Unfortunately, there was no further correlation with the phenotype or the presence of spermatogenesis of the participants in this study [13]. Another study also investigated X-chromosomal origin and found a paternal origin in 26/61 (43%) of cases and a maternal origin in 35/61 (57%) cases [9]. They reported a higher incidence of speech and language problems and motor impairment of men with KS who had a paternally inherited extra X-chromosome [9]. The group with the paternal extra X-chromosome also had increased body size parameters such as sitting height and penile length [9]. Another study reported X-chromosomal inheritance in 41 men with KS, of whom 20/41 (49%) had a paternal extra X-chromosome and 21/41 (51%) had a maternal extra X-chromosome [14]. In contrast to the study by Stemkens et al. (2006), there was no difference between the two groups observed in learning disabilities and cognitive performance. Furthermore, they found no difference regarding education, personality and autism traits, psychological distress and socioeconomic factors [14]. Therefore, the impact of the inheritance pattern of the supernumerary X-chromosome in men with KS remains to be unclear. Comparing to our results and others, it seems that the inheritance of the additional maternal or paternal X-chromosome is probably a random process. Nevertheless, inactivation of some key genes on either the maternal or paternal X-chromosome could be responsible for the presence of spermatogenesis, as about 10% of the genes on the X-chromosome are expressed in the testis [10]. It has been shown that 20% of the genes on the second X-chromosome are randomly inactivated; another 15% escape inactivation and 65% are always inactivated [15]. The presence or absence of spermatogenesis might depend on a specific maternal or paternal expression or inactivation for each of the genes being expressed in the testis. However, to further investigate this hypothesis extensive genetic studies with sufficient participants would be necessary to detect a specific expression pattern within the cluster of these many genes. The results of our analysis on spermatogenesis show that for some KS patients, immature germ cells can be found by histology or cytology, while the TESE was negative for spermatozoa. Our finding is supported by several earlier studies, also reporting the presence of spermatogenesis and a successful repeated TESE in men with KS who had a negative TESE at first [16,17]. This apparent discrepancy supports the hypothesis that there are small patches of functioning germ cells and support cells present in men with KS. Despite degeneration and hyalinisation of the testicular environment, there still seem to be reserves of functional germ cells present in some men with KS [18]. Neither hormone levels nor age appeared to be a significant prognostic marker for spermatozoa retrieval or the presence of spermatogenesis in our study population.

Conclusion

The maternal or paternal origin of the second X-chromosome in men with KS does not predict the presence of spermatogenesis.

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Conflict of Interests

S Franik, D Smeets, G van de Zande, I Gomes, K D'Hauwers and L Ramos declare to have no conflict of interest. Dr. Braat reports unrestricted grants from Merck Serono, Ferring and MSD, outside the submitted work. Dr. Fleischer reports personal fees from Merck Serono and Ferring, and unrestricted grants from Ferring and Merck Serono outside the submitted work.

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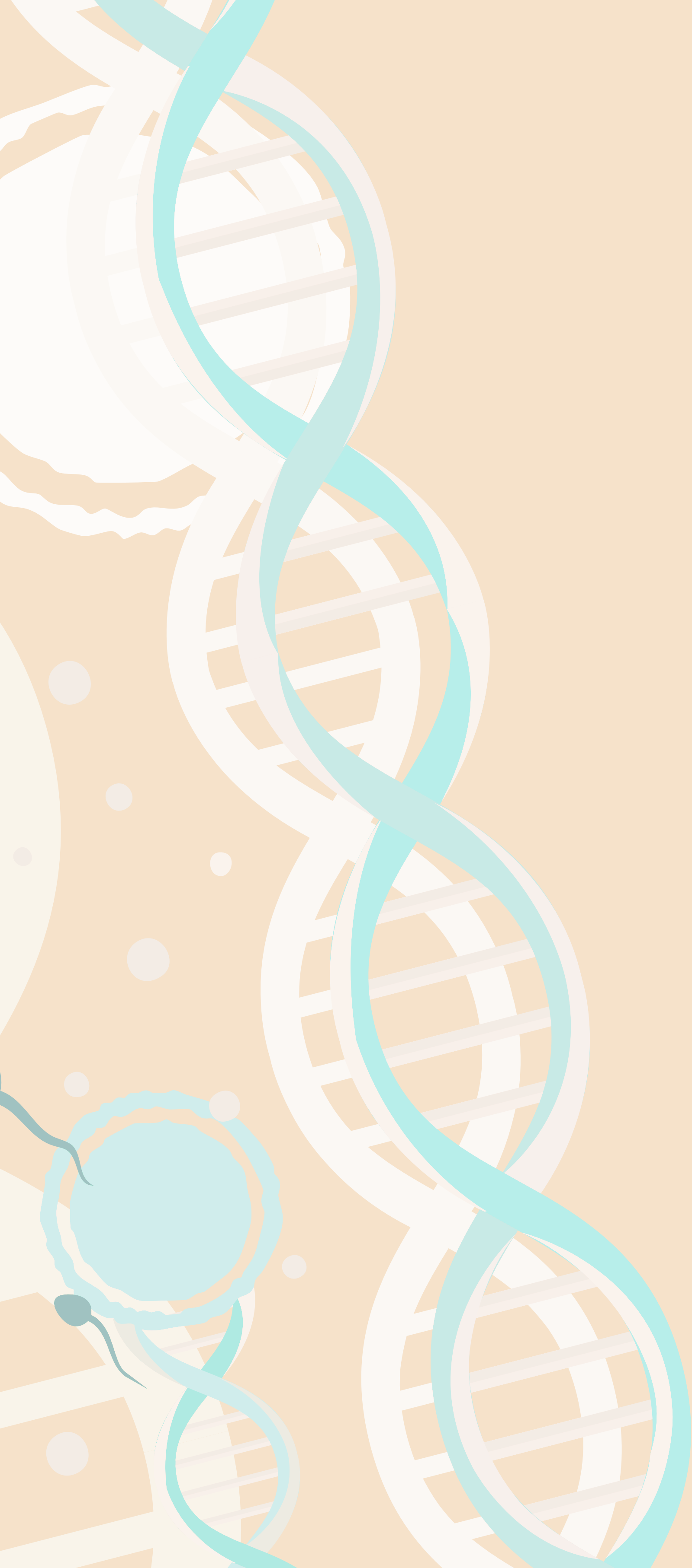

Chapter IV

Healthcare

Evaluation of healthcare experiences in men with Klinefelter Syndrome – a multicentre study

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Abstract

Introduction: Despite its high prevalence, there are still challenges in diagnostics and accessing specialized care for men with Klinefelter syndrome (KS), a genetic disorder characterized by one or more additional X chromosomes in males. This study investigates satisfaction with healthcare among men with KS.

Methodology: Data from 205 adult males with a 47,XXY karyotype from 16 centres in 6 countries were analyzed, focusing on satisfaction with healthcare services, utilization patterns, and factors influencing satisfaction. A multidisciplinary approach was employed to assess various aspects of care quality, including satisfaction with diagnostic processes, patient-centered care, and doctors' behavior.

Results: Out of the 205 participants, 65.4% reported having a regular general physician and 66.8% consulted one or more specialists trained in KS. However, challenges in finding and scheduling appointments with specialists were noted, with 39.5% reporting difficulties to find a specialist and 29.5% experiencing difficulty in scheduling appointments. Moreover, 50.3% of respondents did not receive any KS-related medical support during childhood or adolescence, as most of them had not received their diagnosis yet. Among those who did, the majority had moderate to high levels of satisfaction with healthcare during their adolescence. Satisfaction levels had a positive correlation with a better health status.

Conclusion: Despite moderate to high satisfaction with healthcare, challenges persist in accessing specialized care and early diagnosis for males with KS. The findings underscore the importance of addressing barriers to care and improving access to multidisciplinary support. Early intervention strategies and increased awareness among healthcare providers are crucial to enhancing care quality and optimizing health outcomes for individuals with KS.

Introduction

Klinefelter syndrome (KS), a genetic disorder characterized by one or more additional X chromosomes in males, results in a 47,XXY or higher-grade X aneuploidy karyotype [1-3]. This condition is one of the most prevalent sex chromosome aneuploidies, with an incidence of approximately 1 in 500 to 1,000 live male births [4]. Despite its prevalence, only about 25% to 40% of males with KS are diagnosed during their lifetime [4-6].

A significant challenge in managing KS is delayed diagnosis due to the large variety in symptoms and lack of awareness among healthcare professionals [7,8].

Individuals with KS often face unique learning challenges and educational difficulties, including language delays, reading and writing difficulties, and attention deficits [9-13]. Klinefelter men seem less accurate in perception of socio-emotional cues such as angry facial expressions; they are less able to identify and verbalize their emotions, but experience increased levels of emotional arousal, in comparison to the general population [14]. It is crucial to provide educational support and specialized learning strategies tailored to their unique needs [10,15].

Adequate testosterone supplementation can improve physical manifestations such as libido, muscle mass, and bone density and also has positive effects on psychological well-being and quality of life [15-17].

Infertility is a prevalent issue in KS that often leads to a diagnosis during adulthood [18]. Affected individuals often have reduced sperm production or azoospermia [19]. Advances in assisted reproductive technologies, such as testicular sperm extraction (TESE) and in vitro fertilization (IVF), have provided opportunities for individuals with KS to achieve biological parenthood [20]. However, access to fertility preservation services and assisted reproductive technologies may be limited, and the associated costs can be prohibitive. Ensuring equitable access to fertility preservation options and providing evidence-based counseling and support for family planning are crucial aspects of comprehensive healthcare for individuals with KS [21].

Psychosocial support and mental health care are other important aspects of the comprehensive care for men with KS [22]. They often grapple with a spectrum of psychosocial challenges, including anxiety, depression, social difficulties, and self-esteem issues, which can significantly impede their overall well-being and quality of life [23,24]. Therefore, it is essential to provide adequate psychosocial support and mental health care, encompassing counseling services, support groups, and interventions tailored to address their unique social and emotional difficulties [25].

The management of KS necessitates a multidisciplinary approach, involving a team of healthcare professionals from various specialties, including endocrinologists, geneticists, urologists, psychologists, speech therapists, and educators [12,22]. Each specialist contributes their expertise to holistically address the diverse medical and psychosocial needs associated with KS. Integrated care models have been proposed as solutions to enhance healthcare delivery by fostering coordinated efforts among professionals [26,27]. One example is the eXtraordinarY Kids Clinic in the US, presenting such a multidisciplinary approach for children with sex chromosome aneuploidies [28].

Despite advancements in our understanding of KS and improvements in healthcare delivery models, gaps still exist in providing optimal care for this population. The aim of this article is to evaluate current healthcare, investigate satisfaction with care, and to identify factors that are associated with satisfaction with care. This international multicentre study includes exploring the quality of healthcare, the effectiveness of current treatment approaches, and the impact of these on the quality of life of individuals with KS. Our goal is to provide insights that can guide future research and inform clinical practice to improve the health outcomes and quality of life of individuals with KS.

Methods

Study population

This study was part of the European dsd-LIFE study (https://www.dsd-life.eu/), a non-interventional, clinical, observational cross-sectional study [29]. The purpose of the dsd-LIFE study was to investigate and compare the long-term outcomes of surgical and hormonal therapy and psychological and social support in adolescents and adults with different forms of disorders of sex development (DSD), aiming to provide the basis for improvements in evidence-based recommendations for care. Ethical approval was given by all institutional ethical boards of the participating centers and informed consent was provided by all participants.

The dsd-LIFE consortium consists of 14 European centres in 6 European countries, i.e. Germany, France, the Netherlands, Poland, Sweden and the United Kingdom

(UK). The 14 centers approached former and current patients by mail, e-mail, phone or direct contact of the physician and promoted participation in patient support groups. Details on the theoretical and methodological framework of the dsd-LIFE study have been published earlier [29].

Men with KS were asked to fill out a digital Patient Reported Outcome (PRO) form that comprised validated and self-constructed questionnaires on health status, mental health, quality of life, psychological well-being, psychosexual outcome, testosterone treatment, fertility, experiences with care and sexuality. To ensure confidentiality, the participants were asked to fill out the PRO with a secure password either in the clinic or at home. Data were entered anonymously into a database. To ensure a homogenic KS study population, only men with a 47,XXY genotype were included in the analysis. Men with KS with a mosaicism or a different genotype (eg. 48,XXXY) were excluded from analysis.

Description of outcome variables

For evaluation of overall satisfaction with quality of care, an adapted version of the Client Satisfaction Questionnaire (CSQ4), was used. The CSQ4 questionnaire is a multiculturally validated questionnaire, evaluating consumer satisfaction with health and human services including governmental and public benefit programs and services [30]. The CSQ4 was developed for cross-cultural comparisons of consumer satisfaction and is available in more than 50 languages, including all dsd-LIFE languages [30]. The CSQ4 contains four questions with each a 4-point-scale to answer ranging from 1 (strongly dissatisfied) to 4 (strongly satisfied). Higher scores indicate a higher satisfaction with care.

For further evaluation of current care and support, the Child Health Care measure satisfaction, utilization and needs (CHC-SUN) questionnaire was used. The first part of the CHC-SUN consists of 10 open questions, investigating the necessity, availability and satisfaction with a general practitioner, a specialist and a psychologist trained in health needs specific for men with KS. Each question has been reported separately, using descriptive statistics such as frequency, means and standard deviations.

The second part of the CHC-SUN is a comprehensive and validated instrument that covers multiple domains of health, including diagnosis/information, patient-centred care, doctor’s behavior. The questionnaire consists of 9 items, with 3 items per domain. Participants were instructed to respond to each item by marking their level of agreement or frequency on a 5-point Likert scale, ranging from 1 (strongly disagree/never) to 5 (strongly agree/always). Descriptive statistics, such as means and standard deviations, were computed to summarize the participants' responses for each domain of the CHC-SUN questionnaire and then transformed to a scale from 0 to 100 to enable comparisons between domains. Higher scores indicate a higher quality of life. A total sum score of all domains has also been calculated and transformed to a scale from 0 to 100.

Possible associated factors

Factors that may influenced quality of life (QOL) that have been investigated are body mass index, subjective general health, social activities, presence of a chronic health problem, household income, testosterone treatment and age at diagnosis; The variables are described in more detail in table 1.

Table 1 Questions used to evaluate possibly associated factors of satisfaction with healthcare in men with KS.

Possible associated factors	Classification/question	Survey	Answering options
Subjective general health	'How is your health in general?'	ESS	• Very good (5) • Good (4) • Fair (3) • Bad (2) • Very bad (1)
Social activities	'Compared to other people of your age, how often would you say to take part in social activities?'	ESS	• Much more than most (1) • More than most (2) • About the same (3) • Less than most (4) • Much less than most (5)
Presence of chronic health problems	'Do you have any longstanding illness or health problem? (apart from your condition)'	SC	• Yes (1) • No (0)
Physical or mental health problem	'Is this a physical health problem (e.g. Diabetes, coronary heart disease) or a mental health problem (e.g. depression, eating disorder)'	SC	• Physical health problem (1) • Mental health problem (2) • Both (3) • I don't know (0)
Testosterone supplement	'Are you on Testosterone therapy at present?'	SC	• Yes (1) • No (0)
Age at diagnosis	'At what age was your condition diagnosed?'	SC	• Before birth • At birth (0 – 1 month) • Infancy (1 month – 3 years) • Childhood (4 – 12 years) • Adolescence (13 – 17 years) • Adulthood (≥18 years) • I don't know

ESS = European Social Survey question. SC = self-constructed question.

Statistical analysis

Characteristics of the men with KS are described using means and standard deviations (SD) or frequencies and percentages.

Linear regression analysis was done within the KS study population to investigate associations between satisfaction with healthcare and the above described possibly associated factors, such as hormone therapy with testosterone, age at diagnosis, and presence of chronic health problems amongst men with KS. There was no correction for multiple comparisons because of the exploratory nature of this study and the primary concern about type II error. For statistical analyses SPSS software was used (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp).

Results

Basic characteristics of the KS study population

A total of 218 men with KS were available from the dsd-LIFE study, but thirteen men with KS were excluded from the current analysis due to mosaicism or more than one additional X-chromosome. A total of 205 men with KS had a 47,XXY karyotype and were included in the analysis. The baseline characteristics of men with KS are listed in table 2. These men with KS were diagnosed at various life stages: KS was diagnosed in 11/205 (5.4%) men prenatally, in 52/205 (25.4%) men during childhood/adolescence and in 120/205 (58.5%) during adulthood, for the remaining 22/205 (10.7%) participants the age at diagnosis was unknown.

Table 2 Baseline characteristics of men with Klinefelter syndrome.

	Participants with Klinefelter Syndrome (n=205)
Age in years, Mean (SD), range	39.9 (15.0), 15-75
Height in cm, Mean (SD)	184.0 (12.5)
Weight in kg, Mean (SD)	82.6 (27.6)
BMI in kg/m, Mean (SD)	24.6 (6.7)
Country of residence (n/%)	
Germany	36 (17.6%)
France	23 (11.2%)
Netherlands	83 (40.5%)
Poland	23 (11.2%)
Sweden	32 (15.6%)
United Kingdom	8 (3.9%)
Testosterone supplement at present (n/%)	
Yes	145 (70.7%)
No	9 (4.4%)

Table 2 Continued

	Participants with Klinefelter Syndrome (n=205)
Unknown	51 (24.9%)
Age at diagnosis (n/%)	
Prenatal	11 (5.4%)
Childhood	52 (25.4%)
Adulthood	120 (58.5%)
Unknown	22 (10.8%)
Social activities (n/%)	
Much more than most	5 (2.4%)
More than most	17 (8.3%)
About the same	74 (36.1%)
Less than most	61 (29.8%)
Much less than most	26 (12.7%)
Unknown	22 (10.7%)
Presence of chronic health problems (n/%)	
Yes	109 (53.2%)
No	69 (33.7%)
Unknown	27 (13.2%)
Experienced Discrimination based on condition (n/%)	
Yes	42 (20.5%)
No	139 (67.8%)
Unknown	24 (11.7%)
Member of a group discriminated against, based on… (n/%)	
Colour or race	1 (0.5%)
Language	1 (0.5%)
Ethnic group	1 (0.5%)
Age	1 (0.5%)
Gender	4 (1.8%)
Sexuality	7 (3.2%)
Disability	7 (3.2%)
Other	1 (0.5%)
Subjective general health (n/%)	
Very good	18 (8.8%)
Good	86 (42.0%)
Fair	59 (28.8%)
Bad	23 (11.2%)
Very bad	5 (2.4%)
Unknown	14 (6.8%)
Gynaecomastia (n/%)	
Yes	36 (17.6%)
No	169 (82.4%)
Small testes (n/%)	
Yes	83 (40.5%)
No	122 (59.5%)

Furthermore, 125 individuals (65.4%) out of 191 participants reported having a General Practitioner (GP) who regularly attends to their health needs. When asked about the frequency of visits to their primary care provider over the past 12 months, 1.6% of these 125 individuals reported not having seen their GP at all, while 18.4% reported a single yearly visit. Twice yearly was reported by 13.6%, three and four times a year each 12.0%, while the remaining 42.4% reported five or more visits a year.

Regarding specialist care, 137 out of 191 participants (66.8%) reported seeing one or more specialists trained in their condition. The types of specialists consulted included andrologists (8 individuals), endocrinologists (92 individuals), urologists (18 individuals), and sexologists (3 individuals). Among these 137 individuals, 4.4% reported not having seen the specialist at all in the past year, while 10.9% reported six or more visits. The process of finding a specialist and scheduling an appointment presented varying levels of difficulty for participants. While 60.0% found it not difficult to find a specialist, 70.5% reported the same ease in scheduling an appointment. A smaller proportion of participants, 17% (32 out of 188), sought a psychologist trained in KS. Among these 32 individuals, 28% found it extremely difficult to find such a psychologist, while an equal percentage found it not difficult. When it came to scheduling an appointment with the psychologist, 53% of the 32 men reported no difficulty, while 25% found it extremely difficult.

These findings highlight the varied experiences and needs of men with KS in relation to healthcare services, and underscore the importance of improving access to specialist care and psychological support for this population.

Results from the Customer Satisfaction Questionnaire (CSQ)

Out of the 205 participants, 194 completed the CSQ (94.6%). The CSQ Sum Score ranged from a minimum of 5.0 to a maximum of 16.0, with a mean score of 12.7 (SD = 2.6; median: 13.0) indicating a moderate to high level of satisfaction among men with KS regarding the quality of care they received.

Results from CHC-Sun Questionnaire

A total of 199 participants completed the second part of the CHC-SUN questionnaire to assess different aspects of the quality of care. The CHC Total sum had a mean score of 55.0 (SD = 24.1) out of 100, indicating a moderate level of perceived quality of care. Figure 1 shows the CHC sum scores per country participating in this study.

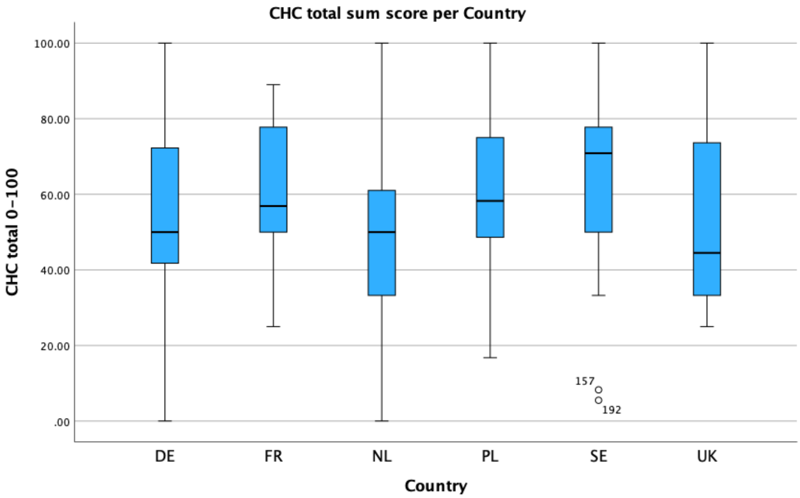

Figure 1 Boxplot of the results of the second part of the CHC-SUN questionnaire: CHC total sum score per country (DE: Germany, n=33; FR: France, n=22; NL: Netherlands, n=70; PL: Poland, n=23; SE: Sweden, n=30; UK: United Kingdom, n=8). The graph shows the median (middle horizontal line within the box), the 25% and 75% percentiles (lower and upper edge of the box), and the range of the sum scores (in the whiskers, where the single points represent outliers).

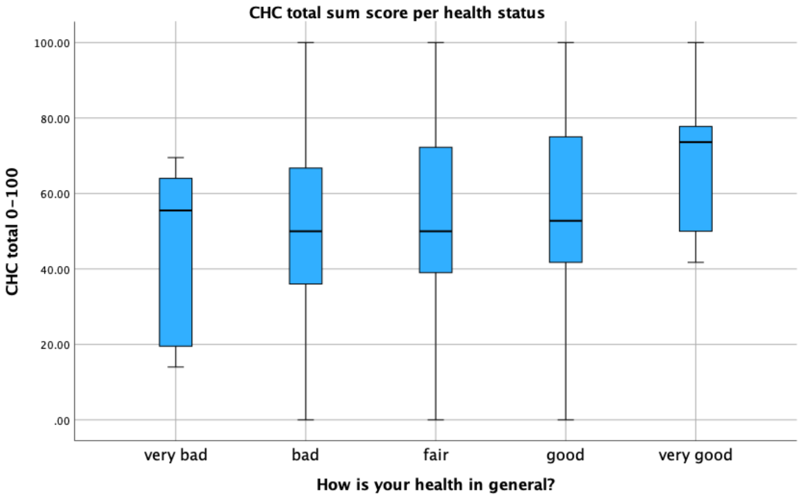

Figure 2 Boxplot showing the results of the second part of the CHC-SUN questionnaire: CHC total sum score per health status (very bad n=5; bad n=22; fair n=57; good n=84; very good n=18). The graph shows the median (middle horizontal line within the box), the 25% and 75% percentiles (lower and upper edge of the box), and the range of the sum scores (in the whiskers). The Spearman correlation coefficient was significant with a p-value <0.05 and a correlation coefficient of 0.17.

Influencing factors

The satisfaction with quality of healthcare was highly dependent on the participants´ individual health.

These findings suggest that individuals who perceive their health as better tend to have higher CHC-SUN scores, with a Spearman correlation coefficient of 0.17 (p-value=0.02). Even though the simple presence of any longstanding health problem was not statistically significantly associated with lower CHC-SUN total scores (p-value=0.36) it was consistent with the correlation found above: no health problem 57.3 (SD 2.9) vs health problem 53.8 (SD 2.3). The average CHC-SUN total score of men with KS who had physical health problems was 58.3 (SD 3.1), the average score of men with KS with mental health problems was 61.5 (SD 10.0), while the average health score of men with KS with both mental and physical health problems was 47.8 (SD 4.7). These findings suggest that individuals who perceive their health as better tend to have higher CHC-SUN scores, and the presence of both mental and physical health problems seems to have a strong negative impact on their perceived general health status.

The participants' perceptions of specific domains of health care, including diagnosis/ information, patient-centred care, and doctor’s behavior were also assessed using the CHC-Sun questionnaire. The Diagnosis sum, which assesses the participants' satisfaction with the diagnostic process, had a mean score of 52.0 (SD 25.0) out of 100. The Patient-centred care sum, which evaluates the participants' perception of care that is tailored to their individual needs and preferences, had a mean score of 52.5 (SD 25.6) out of 100. The participants' responses indicate a moderate level of satisfaction with the diagnosis process and patient-centered care. Regarding the participants' perception of their doctors' behavior, the CHC doctor's behavior sum had a mean score of 59.8 (SD = 26.5) out of 100. The responses suggest a moderate level of satisfaction with their doctors' behavior during the provision of care.

Linear regression analysis

The results of the linear regression analysis are shown in table 3. These findings suggest that subjective general health is significantly positively associated with both CHC-SUN total sum score and the CSQ4 sum score, while older age at diagnosis is significantly associated with a lower CHC-SUN total sum score. Other factors, such as level of education, testosterone substitution, household’s income, and BMI, do not show a significant association with either score.

Table 3 Results of linear regression analysis of the CHC-SUN and CSQ4 total scores with possibly associated factors in our study population of men with KS (n=194). ANOVA was used to test for statistical significance.

	Independent variable	**Regression coefficient** (B)	**Coefficient's Std. Error**	**Standardized coefficient** (beta)	**P-value**
CHC-SUN total sum score (scale: 1-100)	Level of Education (low-medium-high)	0.19	0.13	0.11	0.13
	Age at Diagnosis (years)	-0.05	0.16	-0.03	0.74
	Testosterone substitution received; yes (1) / no (0)	-1.62	3.03	-0.04	0.59
	Subjective general health (very bad (1) – very good (5))	4.69	1.90	0.18	0.01
	Household's income (low (1) – high (4))	0.05	0.11	0.03	0.66
	BMI (kg/m^2)	-0.26	0.35	-0.09	0.45
CSQ4 (scale: 4-16)	Level of Education (low – medium - high)	0.03	0.01	0.16	0.03
	Age at Diagnosis (years)	-.004	0.02	-0.02	0.82
	Testosterone substitution received (yes/no)	-0.30	0.33	-0.07	0.37
	Subjective general health (1 – 5)	0.57	0.21	0.20	0.01
	Household's income (1 – 4)	.001	0.01	0.006	0.94
	BMI (kg/m^2)	-0.02	0.04	-0.06	0.60

Self-constructed Questionnaire

The second part of the CHC-SUN survey found that a significant number of participants did not receive medical support during their childhood or adolescence, and among those who did, satisfaction levels varied widely (table 4). In adulthood, many individuals reported either receiving support from KS-related groups or believing they would have benefited from such services, though a substantial portion of 44.3% felt they did not need these services (table 4). Similarly, for case manager services, a minority received them or believed they would benefit, while a majority of 63.9% did not see a need for them. These findings highlight the diverse experiences and needs regarding medical and support services among men with KS.

Table 4 Second part of the CHC-SUN survey about medical support and support groups of men with KS (n=205).

Medical Support During Childhood / Adolescence	**Count**	**Percentage**
No medical support	94	45.9%
Received medical support	111	54.1%
Very satisfied	16	14.4%
Satisfied	33	29.7%
Neither satisfied nor dissatisfied	20	18.0%
Dissatisfied	14	12.6%
Very dissatisfied	10	9.0%
Support Groups in Adulthood		
Received support	34	18.4%
Partly received support	21	11.4%
Did not receive but believed they would benefit	48	25.9%
Did not need the service	82	44.3%
Case Manager Services		
Received services	11	5.4%
Partly received services	11	5.4%
Did not receive but believed they would benefit	38	18.5%
Did not need the service	106	51.7%
Missing	39	19.0%

Discussion

The purpose of our study was to investigate the satisfaction with healthcare among men with KS and identify factors that may influence this satisfaction. We showed that the satisfaction with health care is moderate and associated with different factors. The factors that are most strongly associated with satisfaction were good accessibility of health care and higher general health scores as indicated by CHC-SUN.

Regarding our study's results, several new findings emerge from the data. Firstly, in terms of healthcare utilization, it is notable that a majority of participants (65.4%) reported having a regular GP who attends to their health needs. However, it is important to realise that 80 participants (40%) did not fill in the question regarding the frequency of GP visits over the past 12 months. A possible explanation could be, that the majority of those men do not visit their GP regularly. Understanding the reasons for the missing data and its potential implications for healthcare utilization among men with KS would be valuable.

Up to 66.8% reported seeing one or more specialists trained in their condition, with relatively low numbers of participants accessing specialists in certain domains, such as sexologists (3 participants).

This may be due to difficulties in finding and obtaining appointments with some subspecialists and psychologists trained in KS. A substantial proportion of participants reported finding it difficult to find a specialist trained with their condition. Similar challenges were observed in scheduling appointments, although the majority reported it was not difficult to secure an appointment. In accordance with previous studies, this highlights the need for comprehensive and multidisciplinary care to address the diverse medical and psychosocial needs of men with KS. Further training of first line caretakers, such as family physicians and psychologists could help to improve access to specialized care and reducing barriers in finding and obtaining appointments, ensuring timely and appropriate support for men with KS [27].

Our findings align with and contribute to the current body of literature in several ways. The moderate to high level of satisfaction with healthcare among men with KS, as indicated by the mean CSQ and CHC-Sun questionnaire scores, echoes the findings of an earlier narrative review of the literature [15]. This review also highlighted the psychosocial impact of KS and emphasized the need for improved healthcare for this group [15]. Consistent with Herlihy et al. (2018), the current study suggests potential gaps in preventive healthcare measures for men with KS. Lower rates of cancer screening and vaccinations among men with KS indicate the need for targeted interventions to ensure appropriate preventive care in this population [31]. They reported lower satisfaction with healthcare access and coordination among men with KS compared to a control group [31]. This suggests a potential discrepancy between the perceived quality of care among men with KS and the general population. Those findings were also confirmed by Forti et al, regarding healthcare utilization, as they also found lower healthcare utilization rates among men with KS compared to the general population [18]. It highlights the importance of addressing the specific healthcare needs and concerns of men with KS to enhance their overall satisfaction and experiences within the healthcare system.

The varying ages at diagnosis, with many individuals diagnosed during adulthood, is a recurring theme in the literature and an early diagnosis may be helpful for timely management of KS [24,25]. In our survey on the satisfaction with medical support received during childhood and adolescence among men with KS, an important finding was that 50.3% of the respondents did not provide a response as they had not received any support during this early period. This reflects the high percentage of men with KS not having received a diagnosis of KS during childhood and adolescence. Among those who did receive support, the levels of satisfaction varied, indicating a need for further investigation into the quality of multidisciplinary healthcare services provided to this population. Two studies by Samango-Sprouse et al. (2015) and Aksglaede et al. (2019) highlight the importance of multidisciplinary care and early intervention for men with KS [12,33]. Access to a multidisciplinary team, including endocrinologists, psychologists, speech and language therapists, and other specialists, positively influenced healthcare outcomes and behavioral phenotypes in men with KS [12,33]. Delays in the diagnosis of KS were reported in the study by Aksglaede et al. (2019), emphasizing the need for improved awareness among healthcare professionals and the development of specialized care pathways for timely diagnosis and management [12]. Future research should focus on understanding the barriers to early support and developing interventions to address them.

Limitations

While the study provides valuable insights into the quality of care and satisfaction levels among men with KS, there are several limitations that should be considered when interpreting the findings: Our study included 205 men with KS recruited in highly specialized centers, which may not be representative of the broader KS population. This may limit the generalizability of the findings. The data collected was self-reported at one moment in time, which may introduce bias. Participants' perceptions of their health and satisfaction with care may be influenced by numerous factors, including their mood at the time of the survey, recent experiences, and individual personality traits. Our study did not include a control group of men without KS. This makes it difficult to determine whether the observed satisfaction levels and quality of care are specific to the KS population or reflective of broader trends in healthcare. Our study's cross-sectional design provides a snapshot of the participants' experiences at a single point in time. It does not capture changes in satisfaction or quality of care over time. Our study found a positive correlation between participants' perceived health status and their satisfaction with care. However, unmeasured factors may also influence these outcomes. For example, individuals with better health may have more positive interactions with healthcare providers, leading to higher satisfaction scores. Finally, the study participants were recruited at high level specialized care centers, therefore the evaluation of the quality of care may be overly positive due to a selection bias.

Conclusion

This study sheds light on healthcare experiences among men with KS, revealing both strengths and areas for improvement. While satisfaction levels are generally moderate to high, challenges in accessing specialized care and early diagnosis persist. Addressing these challenges requires a multidisciplinary approach, early intervention strategies, and increased awareness among healthcare providers. Future research should focus on developing tailored interventions to improve access to care and enhance the quality of life for men with KS. By addressing these issues, healthcare systems can better meet the unique needs of men with KS and optimize their overall health outcomes and well-being.

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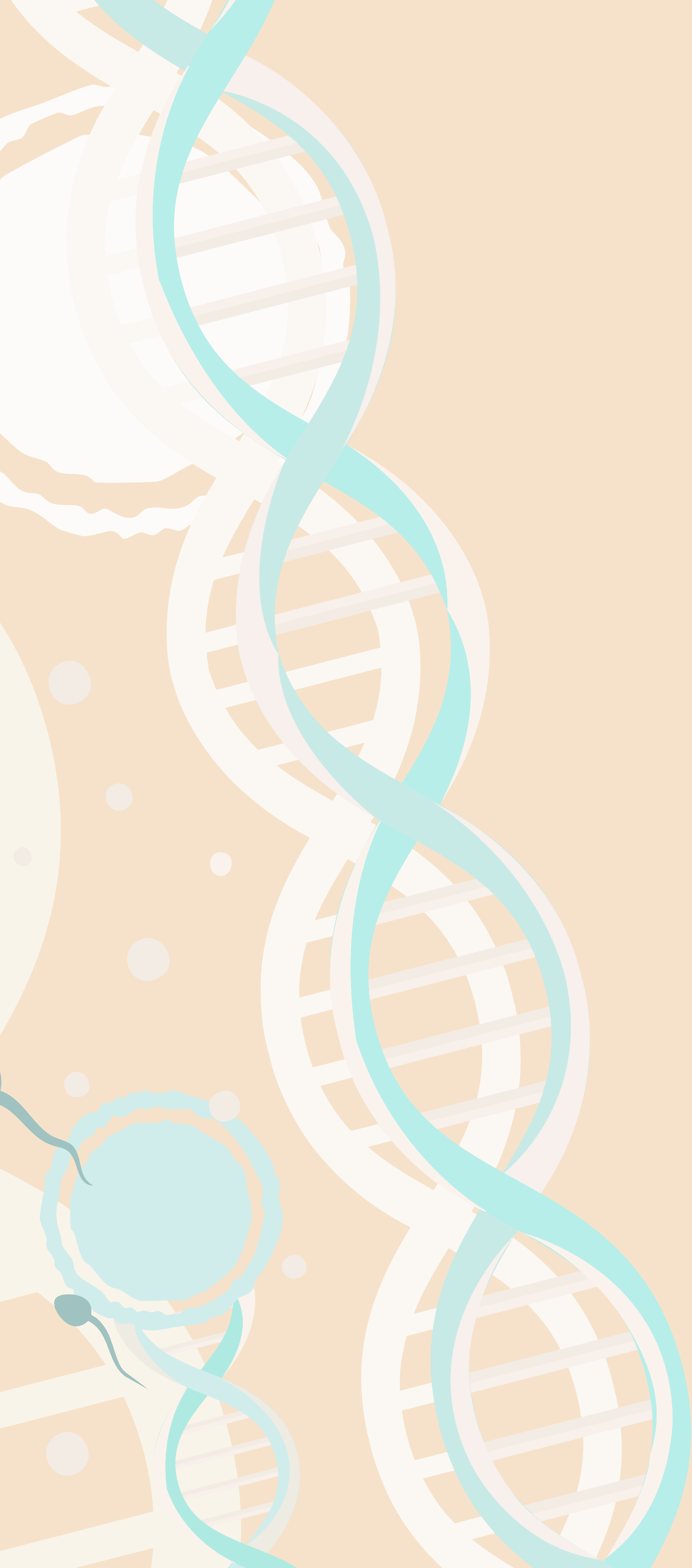

Chapter V

Quality of Life

Quality of life in men with Klinefelter Syndrome – a multicentre study

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Abstract

Background: Klinefelter syndrome (KS) is associated with an increased risk of lower socioeconomic status and a higher risk for morbidity and mortality, which may have a significant impact on quality of life (QOL). The objective of this study is to investigate QOL in a large European cohort of men with KS.

Design: Cross-sectional multicenter study

Methods: Two-hundred-eighteen men with KS were recruited from 14 clinical study centres in 6 European countries which participated in the European dsd-LIFE study. Male normative data from a healthy and a psychiatric reference population were used for comparison. The validated WHOQOL-BREF questionnaire was used to investigate five main domains of quality of life (WHOQOL): Global, physical, psychological, environment and social.

Results: The quality of life (QOL) physical domain score was lower for men with KS compared to the healthy reference population (KS: 66.9; SD19.4, n=193; healthy reference population: 76.5; SD 16.2, n=1324, $p<0.001$) but higher compared to the psychiatric reference population (54.6; SD 20.6; n=77, $p<0.001$). The WHOQOL-psychological domain score was lower for men with KS compared to the healthy reference population (KS: 63.6; SD 17.8, n=193; healthy reference population: 67.8; SD 15.6, n=1324, $p<0.05$) but higher compared to the psychiatric reference population (45.9; SD 26.0), n=77, $p<0.001$). The WHOQOL-social domain score was lower for men with KS compared to the healthy reference population (KS: 60.0; SD21.6, n=193; healthy reference population: 68.2; SD 13.8, n=1324, $p<0.001$) but comparable to the psychiatric reference population (61.0; SD 17.0, n=77, $p=0.5$). The WHO environment domain score of men with KS (70.0; SD 15.0, n=193) was similar to the healthy reference population (70.5; SD 20.7, n=1324) but higher compared to the psychiatric reference population (61.9; SD 20.8, n=77, $p=0.002$). Experienced discrimination, less social activities and the presence of chronic health problems were associated with significantly decreased QOL in men with KS.

Conclusion: Overall QOL in European men with KS is significantly worse compared to a healthy European reference population. Especially the presence of discrimination, less social activities and chronic health problems is associated with lower physical, psychological and social QOL. Further studies are necessary to investigate if a multidisciplinary approach may help to provide adequate counseling and psycho-social support to improve quality of life.

Introduction

With a prevalence of 1 in 660 males, Klinefelter Syndrome (KS, 47XXY) is one of the most common sex chromosome disorders [1]. KS is associated with various morbidities and challenges for affected men, yet it is highly underdiagnosed most likely due to a huge variance in phenotype [1]. There is a higher risk for morbidity amongst men with KS due to somatic disease and mental illness, especially cardiovascular, nervous system, endocrine, metabolic and respiratory disease and mental disorders such as psychoses, disorders of personality and mental retardation [2,3]. A large study in a Danish and British cohort showed a 50% increase in mortality and a 70% increase in risk for hospital admission for men with KS compared to an age matched control group drawn randomly from the Danish civil register [4]. A higher degree of physical impairment and lower levels of subjective general health of men with KS is also associated with a lower socioeconomic status [5]. Psychosocial well-being, which included subjective well-being, self-esteem, body image and psychological distress, was shown to be significantly inferior in a cohort of 87 patients with KS when compared to a general reference population [6]. Another study in a small cohort of 43 boys with KS reported also lower psychosocial health scores, including QOL, low self-esteem, a poor self-concept, and risk for depression [7]. Men with Klinefelter Syndrome might have a higher risk of experiencing discrimination due to their physical, developmental and hormonal differences, such as absence of sexual characteristics, reduced muscle tone, gynecomastia, and sparse facial and body hair [1]. These differences can lead to misunderstandings, stigmatization, and stereotypes about masculinity, undermining their sense of identity and self-worth. Additionally, lack of knowledge among health care providers can result in limited social support, inadequate healthcare services, and challenges in accessing appropriate interventions and accommodations. Men with KS may also face discrimination due to the absence of inclusive policies, accommodations, or resources that address their unique needs.

However, little is known about the quality of life (QOL) in men with KS. Therefore, the objective of this study is to investigate QOL in a large European cohort of men with KS and to associate QOL with social activities, age at diagnosis, hormonal substitution, presence of chronic health problems and experienced discrimination.

Methods

Study population

This study was part of the European dsd-LIFE study (https://www.dsd-life.eu/), a non-interventional, clinical, cross-sectional study [8]. The purpose of the study was to investigate and compare the long-term outcomes of surgical and hormonal therapy and psychological and social support in adolescents and adults with different forms of disorders of sex development (DSD), aiming to provide the basis for improvements in evidence-based recommendations for care. Ethical approval was first sought from the medical ethics committee at the Charité Universitätsmedizin Berlin. Ethical approval was given by all institutional ethical boards of the participating centers and informed consent was provided by all participants.

The dsd-LIFE consortium consists of 14 European centres in 6 European countries, i.e. Germany, France, the Netherlands, Poland, Sweden and the United Kingdom (UK). The 14 centers approached former and current patients by mail, e-mail, phone or direct contact of the physician and promoted participation in patient support groups from February 2014 till September 2015. Participants

had to be at least 16 years old with a medically confirmed clinical and/or genetic diagnosis. Details on the theoretical and methodological framework of the dsd-LIFE study have been published earlier [8].

Men with KS were asked to fill out a digital Patient Reported Outcome (PRO) form that comprised validated and self-constructed questionnaires on health status, mental health, quality of life, psychological well-being, psychosexual outcome, testosterone treatment, fertility, experiences with care and sexuality. To ensure confidentiality, the participants were asked to fill out the PRO with a secure password either in the clinic or at home. Data were entered anonymously into a database.

Reference population

We used a healthy (n=1324) reference population as well as a psychiatric control group (n=77) reported by Skevington et al., 2012, for comparison of the mean scores for the World Health Organization Quality of Life – BREF (WHOQOL-BREF) domain scores [9]. The reference populations were recruited at 38 UK sites in community, primary care, outpatient, inpatient, rehabilitation settings and social care. The healthy reference population included six samples of university students and student nurses, where persons with health conditions were excluded. The psychiatric population contained people with different psychiatric diagnoses, for example depression or schizophrenia [9].

Description of outcome variables

For evaluation of quality of life, a short version of the WHO-QOL-100 questionnaire, the WHOQOL-BREF questionnaire, was used. The WHOQOL-BREF questionnaire is a multiculturally validated questionnaire, evaluating quality of life (QOL). The WHOQOL-BREF was developed for cross-cultural comparisons of QOL and is available in more than 40 languages, including all dsd-LIFE languages (10). Used in equal or similar cultural contexts like in-between Europe, national weightings are not needed in analyses (11). Five QOL main domains were investigated: Global (2 questions), Physical health (7 questions), Psychological (6 questions), Social relationships (3 questions) and Environment (8 questions). It is validated for persons aged 18 years and older (10). All answers are given on a five-point-Likert scale, summed per domain, and then transformed to a scale from 0 to 100 to enable comparisons between domains. Higher scores indicate a higher quality of life. Domains are not scored when two or more items are missing (or 1-item in the 3-item domain social relationship). The WHOQOL-BREF has no overall score. The domains show good psychometric properties without ceiling or floor effects and an internal consistency with Cronbach's alpha being ≥0.8 for every domain, except for social relationships with 0.68 (10,12).

Table 1 Questions used to evaluate possible associated factors in men with KS.

Possible associated factors	Classification/question	Type	Answering options
Subjective general health	'How is your health in general?'	ESS	• Very good • Good • Fair • Bad • Very bad
Social activity	'Compared to other people of your age, how often would you say to take part in social activities?'	ESS	• Much more than most • More than most • About the same • Less than most • Much less than most
Presence of health problems	'Do you have any longstanding illness or health problem? (apart from your condition)'	SC	• Yes • No
Discrimination	'Have you been discriminated against because of your condition?'	SC	• Yes • No
Testosterone supplement	'Are you on Testosterone therapy at present?	SC	• Yes • No

Table 1 Continued

Possible associated factors	Classification/question	Type	Answering options
Age at diagnosis	'At what age was your condition diagnosed?'	SC	• Before birth • At birth (0 – 1 month) • Infancy (1 month – 3 years) • Childhood (4 – 12 years) • Adolescence (13 – 17 years) • Adulthood (≥18 years) • I don't know

ESS = European Social Survey question. SC = self-constructed question.

Possibly associated factors

To ensure a homogenic KS study population, only men with a 47,XXY genotype were included in the analysis. Men with KS with a mosaicism or a different genotype (eg. 48,XXXY) were excluded from analysis. Possibly with QOL associated factors that have been investigated are BMI, social activities, presence of a chronic health problem, experience of discrimination based on condition, experience of discrimination based on various reasons, testosterone treatment and age at diagnosis; they are described in more detail in Table 1.

Statistical analysis

Characteristics of the men with KS are described using means and standard deviations (SD) or frequencies and percentages.

Linear regression analysis was done within the KS study population to investigate possible associations between QOL and the above described possibly associated factors, such as hormone therapy with testosterone, age at diagnosis, and presence of chronic health problems amongst men with KS. There was no correction for multiple comparisons because of the exploratory nature of this study and the primary concern about type II error. Domain scores of the WHOQOL-BREF for men with KS, the healthy and the psychiatric reference population have been compared by unpaired T-tests. Furthermore, a network plot for showing associations between QOL and variables of possible influence was created using Pearson correlation coefficients, restricted to correlation coefficients with a p-value < 0.05. The network plot was created with the SemiPar package (13) using the statistical software R version 4.2.1 (14). For all other analyses SPSS software version 22.0 was used (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp).

Results

Basic characteristics of the KS study population

A total of 218 men with KS were included in the study, but thirteen men with KS were excluded from analysis due to mosaicism or more than one additional X-chromosome. A total of 205 men with KS had a 47,XXY karyotype and were included in the analysis. The baseline characteristics of men with KS are listed in Table 2. KS was diagnosed in 11/205 (5%) men prenatally, in 52/205 (25%) men during childhood/adolescence and in 120/205 (59%) during adulthood, for the remaining 22/205 (11%) men with KS age at diagnosis was unknown. Discrimination based on Klinefelter syndrome was reported to be experienced in 20.5% of men with KS in our study cohort. Discrimination based on other reasons was also investigated, showing very low percentages of discrimination based on ethnicity, language, colour or race (0.5% each) and also low percentages for sexuality (3.2%) or disability (3.2%) (table 2).

Table 2 Baseline characteristics of men with Klinefelter syndrome.

	Participants with Klinefelter Syndrome (n=205)
Age in years, Mean (SD), range	39.9 (15.0), 15-75
Height in cm Mean (SD)	184.0 (12.5)
Weight in kg Mean (SD)	82.6 (27.6)
BMI in kg/m^2 Mean (SD)	24.6 (6.7)
Country of residence (n/%)	
Germany	36 (17.6%)
France	23 (11.2%)
Netherlands	83 (40.5%)
Poland	23 (11.2%)
Sweden	32 (15.6%)
United Kingdom	8 (3.9%)
Testosterone supplement at present (n/%)	
Yes	145 (70.7%)
No	9 (4.4%)
Unknown	51 (24.9%)
Age at diagnosis (n/%)	
Prenatal	11 (5.4%)
Childhood	52 (25.4%)
Adulthood	120 (58.5%)
Unknown	22 (10.8%)

WHOQOL-BREF in men with KS and reference populations

Quality of life global was lower (-15.7) amongst participants who experienced discrimination

The average WHOQOL-BREF global was completed by 193 men with Klinefelter syndrome (KS) and the mean group score was 64.2 (SD 21.7) (figure 1). The score amongst participants who reported to have experienced discrimination based on their condition was significantly lower (-15.7, CI -22.7; -8.7) compared to participants who did not experience discrimination. Furthermore, patient-reported presence of chronic health problems resulted in statistically significantly lower QOL (mean score 57.3, SD 23.4) compared to participants without presence of chronic health problems (mean score 72.5, SD 15.0). Especially mental chronic health problems were associated with a lower quality of life (mean score 45.8, SD 23.3), compared to the presence of physical chronic health problems for men with KS (mean score 61.4, SD 21.5). Furthermore, less participation in social activities was associated with a lower global group score for QOL (Table 3). There was no significant association between global QOL and BMI (p=0.45), current testosterone therapy (p=0.46), and age at diagnosis (p=0.94). Our network plot shows strong positive associations between the QOL global domain and all other QOL domains (figure 2).

Quality of life physical domain was lower (66.9) compared to the healthy reference population (76.5)

The mean WHOQOL-physical domain score of men with KS (n=193) was 66.9 (figure 1). This was significantly lower compared to the healthy reference population (n=1324) who achieved a mean score of 76.5 (p<0.001, figure 1). However, the reference population with psychiatric illness (n=77) scored significantly lower (mean score 54.6) than men with KS (p<0.001) and the healthy reference population (p<0.001, figure 1).

Our univariate analysis shows that the mean QOL physical domain score was significantly lower amongst men with KS who reported to have experienced discrimination based on their KS condition (mean score 57.1) compared to men who did not experience discrimination (mean score 69.0). Furthermore, men with patient-reported presence of chronic health problems had a significantly lower QOL physical domain score (mean score 60.5) compared to men without presence of chronic health problems (mean score 75.3). Less participation in social activities was also associated with lower physical domain scores. There was no significant association between the QOL physical domain score and current testosterone therapy (p=0.83), BMI (p=0.22) or age at diagnosis (p=0.77).

Quality of life psychological health domain was lower (63.6) compared to the healthy reference population (67.8)

The mean WHOQOL-psychological health domain score of men with KS (n=193) was 63.6 (SD 17.8). This was significantly lower compared to the healthy reference population (n=1324) who had a mean score of 67.8 (p<0.001 figure 1). However, the reference population with psychiatric illness (n=77) scored significantly lower than the men with KS and the healthy reference population with a mean score of 45.9 (p<0.001; figure 1). Our univariate analysis shows that the mean psychological health domain score was significantly lower amongst men with KS who reported to have experienced discrimination based on their condition (mean score 56.6) compared to men who did not (mean score 65.0; table 3). Furthermore, men with patient-reported presence of chronic health problems had significantly lower QOL (mean score 60.2) compared to men without presence of chronic health problems (mean score 67.4; table 3). Less participation in social activities was also associated with lower psychological domain scores (table 3). There was no significant association with current testosterone therapy (p=0.06), BMI (p=0.10) or age at diagnosis (p=0.37). (figure 2).

Quality of life social domain was lower (60.0) compared to the healthy reference population (68.2)

The mean WHOQOL-social domain score of men with KS (n=193) was 60.0. This was significantly lower compared to the healthy reference population (n=1324) who achieved a mean score of 68.2 (p<0.001, figure 1). The WHOQOL-social domain score of men with KS was similar to the reference population with psychiatric illness (n=77) who had a mean score of 61.0 (p=0.5, figure 1). The mean social domain score was significantly lower amongst men with KS who reported to have experienced discrimination based on their condition (50.4) compared to men who have not experienced discrimination (mean score 61.9, p=0.008). Less participation in social activities was associated with lower social domain scores (p<0.001). There were no statistically significant associations with self-reported presence of chronic health problems (p=0.26), BMI (p=0.75), testosterone therapy at present (p=0.15) or age at diagnosis (p=0.35).

Quality of life environment domain was similar (70.0) to the healthy reference population (70.5)

The WHOQOL-environment domain score of men with KS (n=193) was 70.0. This was comparable to the healthy reference population (n=1324) who achieved a mean score of 70.5 (p=0.5, figure 1). The reference population with psychiatric illness (mean score 61.9, n=77) scored significantly lower than the men with KS (p=0.002) and the healthy reference population (p<0.001); figure 1). The mean environment domain score was significantly lower amongst men with KS who reported to have experienced discrimination based on their condition (mean score 64.2) compared to men who have not experienced discrimination (mean score 71.4; table 3). Furthermore, the patient-reported presence of chronic health problems was associated with lower scores for WHOQOL-environment (mean score 68.2) compared to men without the presence of chronic health problems (mean score 73.4; table 3). Less participation in social activities was also associated with lower environment domain scores (p<0.047; table 3). There were no significant associations with testosterone therapy at present (p=0.53), BMI (p=0.64) or age at diagnosis (p=0.41).

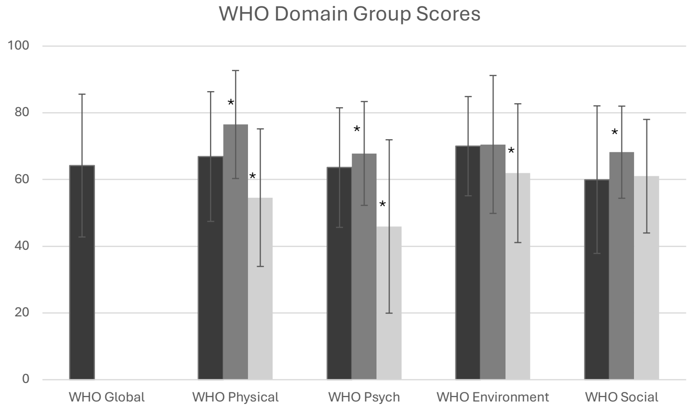

Figure 1 WHOQOL-BREF domain scores (Y axis, 0-100) for men with Klinefelter syndrome from the dsd-LIFE study (black, n=193), for the healthy reference population (grey, n=1324) and the psychiatric reference population (light grey, n=77). * = p<0.05, p-values showing statistically significant differences between men with KS and the reference population based on unpaired two-tailed T-test.

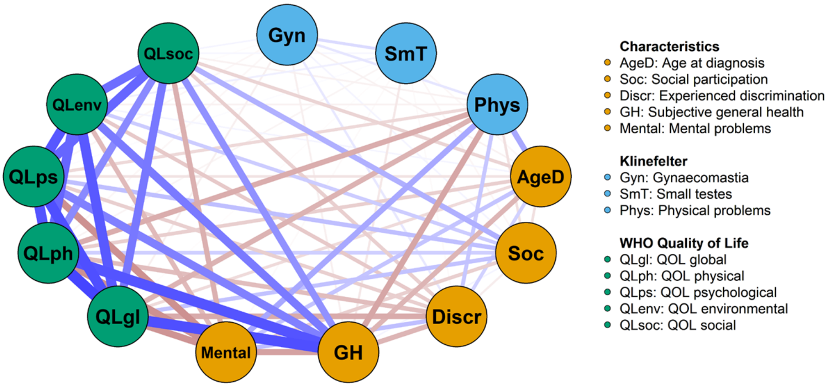

Figure 2 Network plot visualizing Pearson correlation coefficients for various variables of men with KS (n=193). Blue lines represent positive associations, red lines negative associations. A stronger (thicker, darker) line indicates a stronger association between two variables.

Table 3 Results of linear regression analysis of the WHO quality of life domain scores versus possibly associated factors in our study population of men with Klinefelter syndrome (n=193).

	Independent variable	Regression coefficient (B)	95% CI	P-value
WHO Global Group score (scale: 1-100)	Participation in social activities (yes/no)	4.1	0.9; 7.4	0.01
	Age at Diagnosis (years)	-3.8	-12.1; 4.5	0.94
	Testosterone substitution (yes/no)	-1.2	-3.3; 0.9	0.46
	Presence of health problems (yes/no)	-14.1	-20.8; -7.3	<0.01
	Experienced discrimination (yes/no)	-15.7	-22.7; -8.7	<0.01
	BMI (kg/m2)	-0.1	-0.9; -0.7	0.45
WHO physical domain score (scale: 1-100)	Participation in social activities	3.0	0.03; 5.9	0.05
	Age at Diagnosis	-5.5	-13.3; 2.2	0.77
	Testosterone substitution	2.0	-11.6; 15.6	0.83
	Presence of health problems	-13.3	-19.3; -7.4	<0.01
	Experienced discrimination	-12.2	-18.6; -5.7	<0.01
	BMI	0.1	-0.5; 0.8	0.22
WHO psych domain score (scale: 1-100)	Participation in social activities	4.5	1.8; 7.2	0.01
	Age at Diagnosis	-1.6	-9.0; 5.8	0.37
	Testosterone substitution	-7.2	-20.1; 5.6	0.06
	Presence of health problems	-6.2	-12.1; -0.3	0.04
	Experienced discrimination	-7.7	-13.9; -1.6	0.01
	BMI	-0.2	-0.9; 0.5	0.10
WHO environment score (scale: 1-100)	Participation in social activities	2.3	0.03; 4.5	0.05
	Age at Diagnosis	-1.8	-7.8; 4.3	0.41
	Testosterone substitution	-2.1	-12.0; 7.7	0.53
	Presence of health problems	-5.8	-10.5; -1.1	0.01
	Experienced discrimination	-8.4	-13.3; -3.4	<0.01
	BMI	0.1	-0.5; 0.6	0.64
WHO social domain score (scale: 1-100)	Participation in social activities	7.3	4.0; 10.5	<0.01
	Age at Diagnosis	-3.2	-12.6; 6.1	0.35
	Testosterone substitution	-9.9	-25.9; 6.0	0.15
	Presence of health problems	-3.6	-10.8; 3.6	0.26
	Experienced discrimination	-10.5	-18.2; -2.8	<0.01
	BMI	-0.01	-0.9; 0.9	0.75

Discussion

This is the first large European multicenter study comparing quality of life in a large group of men with KS with a healthy UK reference population and a psychiatric reference population. Our study has shown that global QOL in men with KS is significantly lower compared to a UK reference population. The global QOL is lowest amongst those persons with KS who had experienced discrimination during their life or suffer from chronic mental health problems. This highlights the negative impact of discrimination on the overall well-being of men with KS. It is important to promote early support and inclusivity to enhance the quality of life for individuals with KS. This is supported by a previous, smaller study investigating QOL in 43 adolescents with KS, reporting that a poor outcome in QOL directly correlated to the severity of the phenotype, measured as a composite score of physical traits including tall stature, eunuchoid body proportion, wide arm span, large waist circumference, high BMI, small testicular volume, short phallus, or gynecomastia [7]. Furthermore, lower scores compared to a reference population for QOL, self-esteem, body image and mental health were reported in a study using validated questionnaires like the "Personal Wellbeing Index" and the "Rosenberg Self-esteem Scale" among 87 adult men with KS [6]. Herlihy et al. have also shown that age of diagnosis was not a predictor for the presence of a more severe phenotype of KS. In that study the phenotype was measured as a composite score of variables such as testosterone deficiency, breast development, infertility, physical development and learning, behavioral, and communication difficulties. In accordance with these results, there was no significant association between age of diagnosis and QOL in our study population. There was also no significant association between QOL and testosterone supplementation at present in our study. This result should be interpreted with caution because 145 of 205 participants had testosterone supplementation and only 9 men did not take testosterone supplementation, for the remaining participants it was unknown. However, our findings were confirmed by another study, investigating QOL in 132 men with KS in Denmark [15]. In their study, there was also no significant difference between the two KS subgroups with or without testosterone therapy [15]. They also found that men with KS scored significantly lower for both physical and psychological QOL, compared to a matched Danish cohort from the registry. This was confirmed in our study; men with KS scored a significantly lower QOL in the physical, psychological- and social domains compared to the healthy European reference population. This may be explained by the presence of chronic health problems such as psychoses, disorders of personality and mental retardation and problems with participation in social activities [2], but also the fact that men with KS have a higher risk for chronic diseases such as diabetes or heart diseases, which are also associated with lower scores for QOL [2,16,17]. Compared to a European reference population with a diagnosis of various psychiatric illnesses such as depression and schizophrenia, the scores were higher for men with KS in the physical, psychological and environmental QOL domains but not in the social domain. This finding confirms earlier studies indicating that participation in social activities often remains challenging for men with KS [5,18]. Additionally, the lower QOL in the psychological health domain may be explained by the finding that depression and anxiety are often present in men with KS [18]. Potentially contributing factors such as bullying, lower self-esteem issues and social challenges should carefully be evaluated by healthcare providers [19-21].

A novel finding of this study is that men with KS reported less participation in social activities compared to the healthy European reference population and reported lower scores in the quality of life social domain. Their overall quality of life social domain scores were even lower when discrimination based on condition was experienced. Several studies reported higher levels of distress during social interactions, shyness and social anxiety and withdrawal amongst men with KS [22-24]. This may result in less (satisfactory) social activities. In order to improve social skills, men with KS may benefit from early social training and training in coping skills. The need for early social support is emphasized by a study showing that employment status and social support are amongst others the best predictors of psychosocial well-being [6]. Therefore, social engagement and involvement in activities could play a role in enhancing the quality of life for individuals with KS. Promoting social inclusion and providing opportunities for social participation may contribute to improved well-being and reduced discrimination.

Limitations

This is the largest study of men with KS using a validated questionnaire to investigate QOL. There was no matched reference group for our cohort of men with KS, therefore we used published data from a general European reference population with a mean age of the reference population was comparable to our study group. Unfortunately, almost all cases of the control group were from the UK and their BMI was unknown which might be a confounder in the study. A main limitation of this study is the possibility of selection bias, as men with KS were mostly recruited from participating specialized outpatient clinics and from patient support groups [8]. Unfortunately, it is unknown how many possible participants have been contacted at the different recruiting clinics and patient support groups but were not willing to participate. Furthermore, the questionnaire used in this study was rather long, taking about 3 hours to fill in, which has led to incomplete filled in questionnaires and more than 10% missing outcomes for some variables (attrition bias). Furthermore, many questions were dichotomous with “yes” and “no” as possible answers. Another limitation of this study is its retrospective, explorative design and that parts of the questionnaire contained self-constructed questions which were not validated. Furthermore, some medical information such as small testes, gynecomastia and testosterone treatment have been collected using a patient reported survey, which can affect the accuracy of the outcomes. In the linear regression analysis, there was no adjustment for multiple comparisons because of the exploratory nature of this study and the primary concern about type II error.

Conclusion

Overall QOL in European men with Klinefelter Syndrome is significantly inferior compared to a healthy European reference population. Especially the presence of discrimination, less social activities and chronic health problems are associated with lower global, physical, psychological and social QOL. Further intervention studies are necessary to investigate if a multidisciplinary approach may help to provide adequate counseling and psycho-social support to improve quality of life of men with KS.

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promotion of collaboration of clinicians, patients and support groups – aiming to improve clinical care for "differences/disorders of sex development".

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Availability of data and materials

The datasets analyzed during the current study are not publicly available as long as primary analyses for other outcomes of dsd-LIFE are not completed. Afterwards scientific public use files are planned. The data will be made available by the principal investigator upon request to researchers after publication of the primary outcomes described in the grant by the consortium.

Disclosure summary

All authors declare no support from any organization for the submitted work; no relationship with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work. Therefore, the authors declare that they have no competing interests.

Trial registration: German Clinical Trials Register: Registration identification number: DRKS00006072, date of registration April 17th, 2014.

DRKS00006072 (German Clinical Trials Register).

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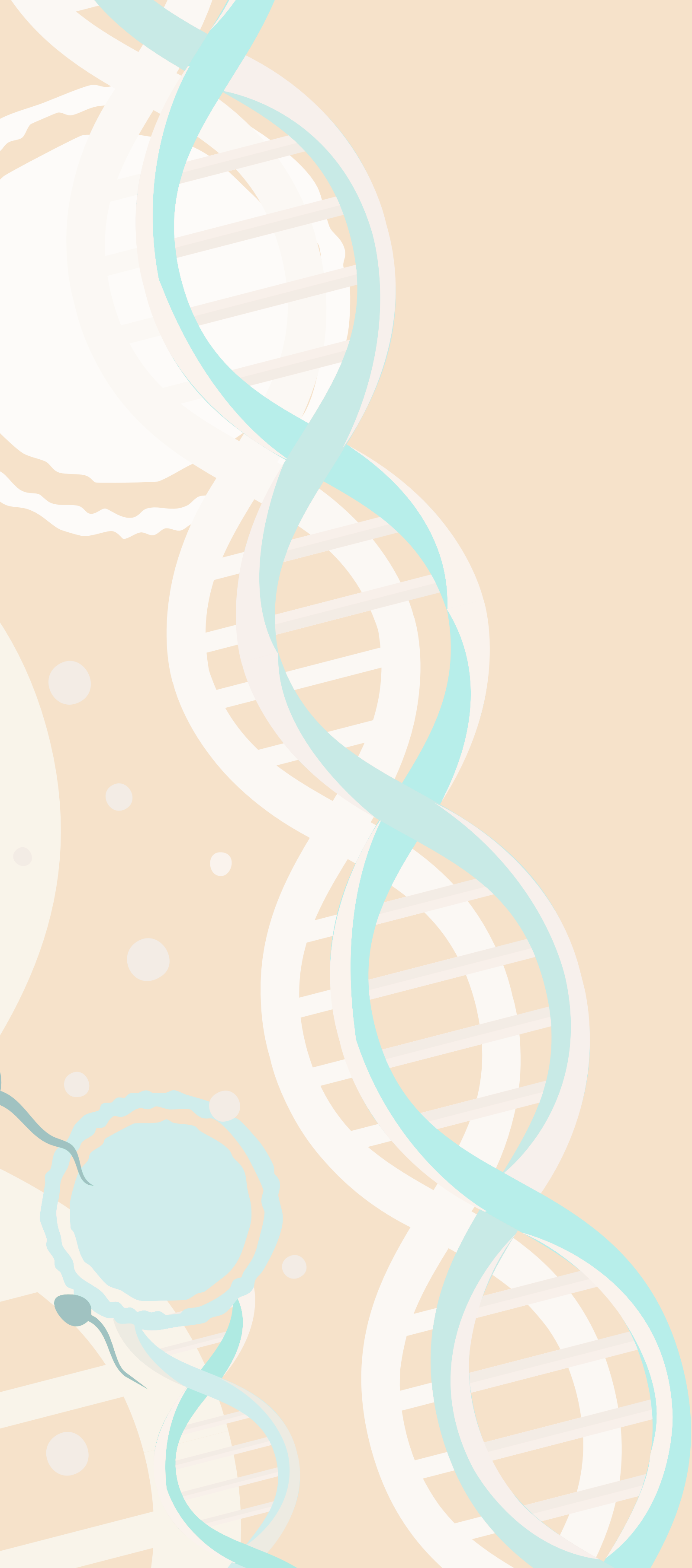

Chapter VI

Socioeconomic status

The impact of Klinefelter Syndrome on socioeconomic status – a multicentre study

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Abstract

Klinefelter syndrome (KS) is associated with an increased risk of neuropsychological morbidity, such as learning disabilities, which may have a significant impact on socio-economic status (SES). The objective of this study was to investigate SES in men with KS, and to associate this outcome with social participation, age at diagnosis, testosterone therapy and physical and mental health status.

Men with KS were recruited in 14 clinical study centres in 6 European countries which participated in the European dsd-LIFE study. Two hundred five men with KS were eligible for inclusion. Male normative data from the European Social Surveys (ESS) were used for comparison.

Data related to education, occupation, satisfaction with income and house holding were collected.

Compared to the ESS reference population, fewer men with KS achieved a high level of education (13% vs 25%, $p<0.001$). There was a significant difference in having a paid job (55% vs 66%, $p<0.001$), and the percentage of absence by sickness or disability was higher amongst men with KS (10% vs 3%, $p<0.001$). Furthermore, satisfaction with current household's income was lower (32% vs 42%, $p<0.01$). Lower scores for subjective general health were associated with lower scores for these outcomes.

Men with KS achieve on average lower levels of education, occupation and report less satisfaction with income compared to the ESS reference population. The presence of health problems and lower scores of subjective general health was related to lower levels of occupation and lower satisfaction with income in men with KS.

Introduction

Klinefelter syndrome (KS), caused by at least one additional X-chromosome in males (47, XXY), is one of the most common numerical chromosomal anomalies in men, with an estimated prevalence of 1-2 per 1000 men [1]. Despite the relatively high prevalence, KS is a highly underdiagnosed syndrome: only about 25% of the affected men are diagnosed, most likely due to a high variation in phenotype [1]. One of the most common medical effects of KS is impaired fertility. Nonobstructive azoospermia is found in about 90% of adult men with KS [1]. Other frequent clinical symptoms include gynecomastia, a low sexual drive, small testes and hypogonadism due to impaired testosterone production [3,4]. KS is also associated with neuropsychological impairment as well as impaired psychological well-being: affected males often suffer from learning disability and social challenges [5,6]. In a web-based survey study, many participants with a late diagnosis reported to have struggled at school, which has led to feelings of inferiority, low self-esteem and depression [6]. Furthermore, men with KS have an increased risk to develop a variety of mental and somatic disorders [7]. Mental and somatic challenges, such as learning disabilities and absence at school or work due to illness, can also lead to lower socioeconomic outcomes during life. Previous studies described inferior outcomes in many different socioeconomic parameters for men with KS compared to the general population such as education, satisfaction with income and social activities [8-10]. A recent study reports an overall lower educational level in Danish men with KS compared to a matched control cohort, leading to a lower income during their lifetime. Additionally, they reported that men with KS retire 16 years earlier on average [11]. The risk of a hospital admission was reported to be elevated by 70% compared to the general population [8]. The life expectancy of men with KS is reduced by 5.6 years compared to the general male population [8].

The objective of this study was to investigate the socioeconomic status (SES), measured by individual education, occupation status and income level, in men with KS and to associate these outcomes with social engagement, age of diagnosis, testosterone therapy and physical and mental health status, and to compare values to a European Social Surveys (ESS) reference population.

Methods

Study population

This study was part of the European dsd-LIFE study, a non-interventional, clinical, cross-sectional study (12). The purpose of the dsd-LIFE study was to investigate and compare the long-term outcomes of surgical and hormonal therapy and psychological and social support in adolescents and adults with different forms of differences/disorders of sex development (DSD), aiming to provide the basis for improvements in evidence-based recommendations for care. Ethical approval was first sought from the medical ethics committee at the Charité Universitätsmedizin Berlin. The study was further approved by all institutional ethical boards of the participating centers and informed consent was provided by all participants.

The dsd-LIFE consortium consist of 14 European centres in 6 European countries, i.e. Germany, France, the Netherlands, Poland, Sweden and the United Kingdom (UK). The 14 centres approached former and current patients by mail, e-mail, phone or direct contact of the physician and promoted participation in patient support groups. Details on the theoretical and methodological framework of the dsd-LIFE study have been published earlier [12].

Men with KS were asked to fill out a digital Patient Reported Outcome (PRO) form that comprised validated and self-constructed questionnaires on health status, mental health, quality of life, psychological well-being, psychosexual outcome, testosterone treatment, fertility, experiences with care and sexuality [12]. To ensure confidentiality, men with KS were asked to fill out the PRO with a secure password either in the clinic or at home. Data were entered anonymously into a database.

Reference population

Normative data on sociodemographic and economic factors were retrieved from the European Social Survey (ESS, http://www.europeansocialsurvey.org).

For our study we used ESS data on health and care, socioeconomic status, family, work and wellbeing, personal and social wellbeing questions from the surveys from 2010 till 2013 including reference data from 1515 participants. We included male participants from United Kingdom, Poland, the Netherlands, Germany, Sweden and France to match this data with our cohort of men with KS. The distribution of participants from the ESS reference population was matched to the distribution of countries and age of participants of the study population using SPSS random matching. For the evaluation of socioeconomic status, the same questionnaires as stated below were used for the ESS reference population.

Description of outcome variables

Standardized validated questions were used in the study- and reference population, as stated in the ESS. For further evaluation of specific aspects of men with KS, self-constructed questions were added in the KS population. The SES consisted of the three following main variables (see Table 1):

- Level of education*:* The international standard classification of education scale (ES-ISCED) is a standardized scale measuring seven levels of education. For parts of the current study this scale was recoded into three groups to compare the outcome to the ESS reference population.
- Occupational status: The standardized ESS question was used to assess occupational status. For Linear regression analysis, the variable was recoded into two categories. The first category was "occupation", containing "in paid work", "in education" and "retired". The other possible answers were regrouped into "no occupation".
- Satisfaction with household income: For the evaluation of satisfaction with household income, the standardized ESS question was used.

Possible associated factors

A subgroup analysis based on the karyotype of study participants was planned but could not be executed due to low numbers of the karyotype. Descriptive analysis of the karyotype (47, XXY; 47, XXY/46, XY; 47, XXY/46, XX; Other; Unknown) was done (see Table 2). Other possible associated factors that have been investigated are summarized in Table 1.

Table 1 Questions used to evaluate socio-economic status and possible associated factors in men with KS and a European reference population.

Subject	Classification/question	Type	Answering options
Socioeconomic status			
Level of education	'What is the highest level of education you have successfully completed'? → Categorized according to the ES-ISCED§: standardized scale that measures education in seven levels.	ESS	ES-ISCED I – V2 Classification: • Low: ES-ISCED I and II • Medium: ES-ISCED IIIb, IIIa and IV. • High: ES-ISCED V1 or V2
	'About how many years of education have you completed, whether full-time or part-time?'	ESS	Number of years

Table 1 Continued

Subject	Classification/question	Type	Answering options
Occupational status	'Which of these descriptions best describes your situation in the last seven days?'	ESS	• In paid work (or away temporarily; employee, self-employed, working for your family business) • In education (not paid for by employer), even if on vacation • Unemployed and actively looking for a job • Unemployed, wanting a job but not actively looking for a job • Permanently sick or disabled • Retired • In community or military service • Doing housework, looking after children or other persons • Other
Satisfaction with income	'Which of the descriptions comes closest to how you feel about your household's income nowadays?'	ESS	• Living very comfortably on present income • Coping • Finding it difficult on present income • Finding it very difficult on present income
Possible associated factors			
Subjective general health	'How is your health in general?'	ESS	• Very good • Good • Fair • Bad • Very bad
Social activity	'Compared to other people of your age, how often would you say to take part in social activities?'	ESS	• Much more than most • More than most • About the same • Less than most • Much less than most
Presence of health problems	'Do you have any longstanding illness or health problem? (apart from your condition)'	SC	• Yes • No
Discrimination	'Have you been discriminated against because of your condition?'	SC	• Yes • No
Testosterone supplement	'Are you on Testosterone therapy at present?	SC	• Yes • No
Age at diagnosis	'At what age was your condition diagnosed?'	SC	• Before birth • At birth (0 – 1 month) • Infancy (1 month – 3 years) • Childhood (4 – 12 years) • Adolescence (13 – 17 years) • Adulthood (≥18 years) • I don't know

ESS = European Social Survey question. SC = self-constructed question. [§]ES-ISCED Classification: I = less than lower secondary; II = lower secondary; IIIb = lower tier upper secondary, IIIa = upper tier upper secondary, IV = advanced vocational, sub-degree; V1 = lower tertiary education, BA level; V2 = higher tertiary education, ≥ MA level.

Statistical analysis

This study embodied three parts:

1) The socioeconomic status (SES) was analyzed in the study population and the reference population and results were compared to each other, using the following outcome variables: level of education, occupation and satisfaction with household income.
2) Within the study population, effects of possibly with SES associated variables such as age at diagnosis, testosterone treatment, social participation and discrimination were further analyzed using linear regression analysis.
3) Furthermore, an exploratory investigation into the relationships between the various variables in men with KS was conducted, based on structural equation modeling (SEM).

The following statistical analyses were done in the three parts of the study:

1) Characteristics of the men with KS and the ESS reference population were described using means and standard deviations (SD) or frequencies and percentages. Main outcome variables (i.e. level of education, occupation status and satisfaction with income) were reported as counts and percentage. For group comparisons between men with KS and the ESS reference population Chi²-tests and Mann-Whitney-U tests were used for the main outcome variables.
2) Linear regression analysis was done within the KS study population to investigate possible associations between the main outcome variables and variables of possible influence, such as hormone therapy with testosterone, age at diagnosis, and presence of physical or mental morbidities amongst men with KS. All analyses were adjusted for age and country of residency. There was no adjustment for multiple comparisons because of the exploratory nature of this study and the primary concern about type II error.
3) Furthermore, an exploratory investigation into the relationships between the various variables in men with KS was conducted, based on structural equation modeling (SEM). SEM is a multivariate technique that requires specification of a model based on theory and research that incorporates both measured variables and latent constructs. The resulting model, based on the best of our knowledge on KS together with model fit criteria, is presented by a path diagram. In addition to the explanatory and dependent variables as mentioned above, a latent variable was included, representing the severity of KS, in an attempt to estimate the (unmeasured) direct impact of the severity of KS. The

latent variable, “Extent of KS”, is based on the variables “gynaecomastia”, “small testes” and “physical health problems”. SEM was performed with maximum likelihood estimation in proc Calis, from the SAS/STAT software, version 9.4 of the SAS System for Windows. SAS is a registered trademark of SAS Institute Inc., Cary, NC, USA. For all other analyses SPSS software version 22.0 was used (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp).

Results

Basic characteristics of the study population

A total of 205 men with KS were included in the study. The baseline characteristics of men with KS and the ESS reference population are listed in Table 2. All 205 included men with KS had a 47,XXY karyotope. Thirteen men with KS had been excluded from analysis due to mosaicism or more than one additional X-chromosome. KS was diagnosed in 63/205 (31%) men during childhood/adolescence and in 120/205 (59%) during adulthood, for the remaining 22/205 (11%) men with KS age at diagnosis was unknown.

Table 2 Baseline characteristics of men with Klinefelter syndrome and the ESS reference population.

	Klinefelter Syndrome (n=205)	**ESS reference population** (n=1515)	**P-value**
Age in years Mean (SD), range	39.9 (15.0), 15-75	40.7 (15.7), 14-75	0.31
Height in cm Mean (SD)	184.0 (12.5)	179.6 (7.7)	<0.01
Weight in kg Mean (SD)	82.6 (27.6)	82.7 (14.4)	0.95
BMI in kg/m^2 Mean (SD)	24.6 (6.7)	26.2 (9.2)	<0.01
Country of residence (n/%)			P=0.94
Germany	36 (17.6%)	251 (16.6%)	
France	23 (11.2%)	184 (12.1%)	
Netherlands	83 (40.5%)	606 (40.0%)	
Poland	23 (11.2%)	167 (11.0%)	
Sweden	32 (15.6%)	268 (17.7%)	
United Kingdom	8 (3.9%)	39 (2.6%)	

Men with KS were significantly taller compared to the ESS reference population: 185.1 (SD 10.0) cm vs 178.8 (SD 7.7) cm; p<0.001. The BMI of men with KS was lower compared to the ESS reference population: 24.6 (SD 6.7) vs 26.2 (SD 9.2); (p=0.01).

Testosterone replacement therapy was given to 145/205 (70.7%) men with KS, more detailed described by Nordenström et al. (14). Unfortunately, no data was available on dosage or duration of testosterone treatment.

1) Socioeconomic status: men with KS versus ESS reference population

The distribution of participants from the ESS reference population was matched to the distribution of countries and age of participants of the study population. The socioeconomic status and possible associated factors of men with KS compared to the ESS reference population are shown in Table 3.

Level of education

Information on education was available for 176 of 205 men with KS. On average, less men with KS achieved a high level of education compared to the ESS reference population (Fig. 1, Table 3). The mean number of years in education was comparable for men with KS and the ESS reference population. A subgroup analysis in men 25 years and older has shown the same results.

Occupational status

Information on current occupation was available for 185 of 205 men with KS. On average, less men with KS had paid work compared to the ESS reference population. The percentage of men being permanently sick or disabled was higher among men with KS compared to the ESS reference population. There were no differences for unemployment, retirement and housework (See Fig. 1, Table 3). A subgroup analysis in men 25 years and older has shown the same results.

Satisfaction with household income

When comparing the overall satisfaction with current household's income, significantly less men with KS reported to live comfortably on their present income (See Fig. 1, Table 3). A subgroup analysis in men 25 years and older has shown the same results.

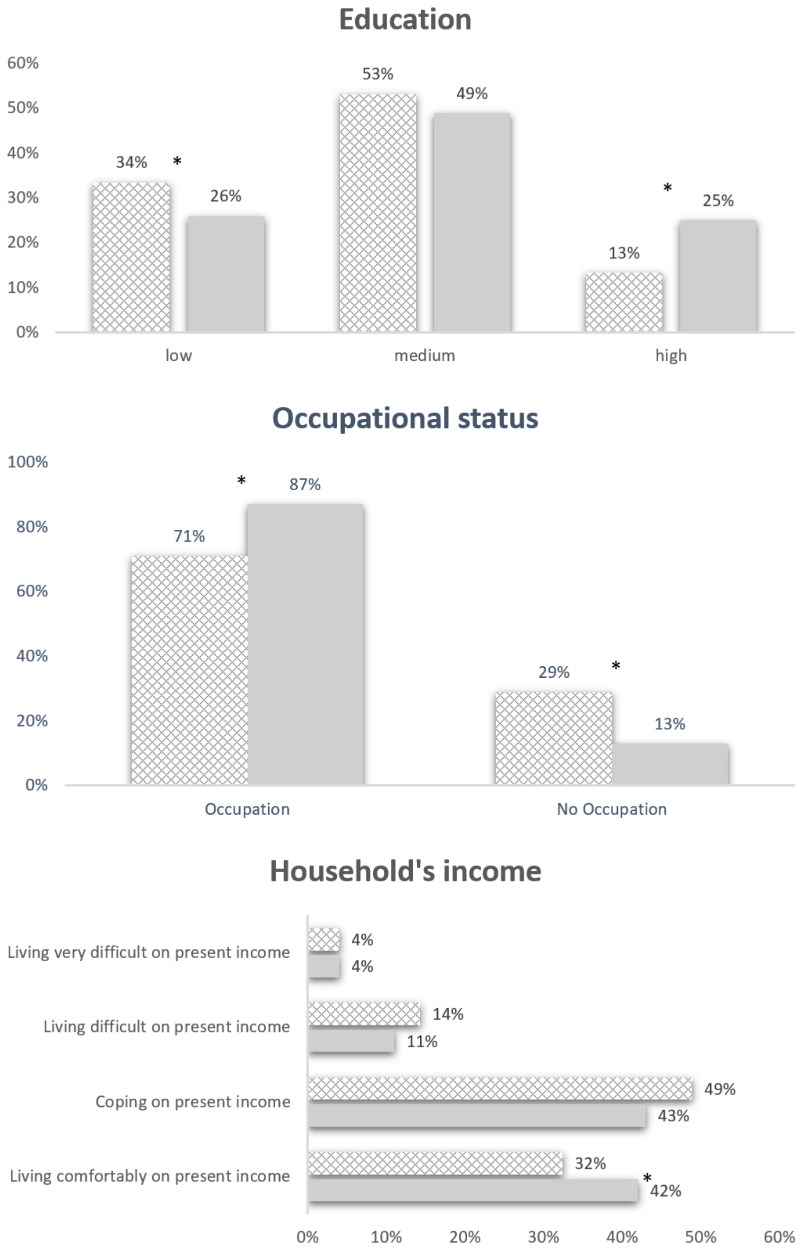

Figure 1 Socioeconomic status of men with KS. Highest level of education received by men with Klinefelter syndrome (squared, n=176) and the ESS reference population (light, n=1512). (*Chi^2, p<0.001). Occupation in men with Klinefelter syndrome (squared, n=185) compared to the ESS reference population (light, n=1513). (*Chi^2, p<0.001). Satisfaction with household income of men with Klinefelter syndrome (squared, n=177) and the ESS reference population (light, n=1515). (*Chi^2, p=0.042).

Table 3 Socioeconomic status of men with Klinefelter syndrome versus a European reference population.

	Participants with Klinefelter Syndrome (n=205)	**ESS reference population** (n=1515)	**P-value**
Level of education (n/%)			P<0.01
High	21 (11.9%)	378 (24.4%)	
Medium	95 (54.0%)	743 (49.3%)	
Low	60 (34.1%)	394 (26.3%)	
Missing	29	3	
Educational years			P=0.06
Median (Q1-Q3)	13.1 (11.0 – 15.0)	13.5 (11.0 – 16.0)	
Occupational status (n/%)			P<0.01
Paid work	103 (55.7%)	1006 (66.5%)	
Education	13 (7.0%)	157 (10.4%)	
Unemployed	18 (9.8%)	102 (6.8%)	
Permanently sick or disabled	19 (10.3%)	50 (3.3%)	
Retired	13 (7.0%)	160 (10.6%)	
Housework/looking after children	1 (0.5%)	24 (1.6%)	
Other	18 (9.7%)	14 (0.9%)	
Missing	19	2	
Satisfaction with income (n/%)			P=0.01
Living comfortably	53 (30.8%)	625 (41.9%)	
Coping	86 (50.0%)	645 (43.2%)	
Difficult	26 (15.1%)	170 (11.4%)	
Very difficult	7 (4.1 %)	52 (3.5%)	
Missing	28	0	
Subjective general health (n/%)			P<0.01
(very) good	104 (50.9%)	1155 (76.6%)	
Fair	57 (27.7%)	293 (19.4%)	
(very) bad	44 (21.3%)	66 (4.4%)	
Missing	0	1	
Social activities (n/%)			P<0.01
(much) more than others	88 (48.1%)	492 (32.7%)	
The same as others	74 (40.4)	730 (48.5%)	
(much) less than others	21 (11.4%)	282 (18.8%)	
Missing	22	11	
Presence of health problems		N/A	
Yes	109 (53.2%)		
No	69 (33.7%)		
Unknown	27 (13.2%)		
Discrimination based on condition		N/A	
Yes	42 (20.5%)		
No	139 (67.8%)		
Unknown	24 (11.7%)		
Testosterone supplement at present		N/A	
Yes	145 (70.7)		
No	9 (4.4%)		
Unknown	51 (24.9%)		
Age at diagnosis		N/A	
Prenatal	11 (5.4%)		
Childhood	52 (25.4%)		
Adulthood	120 (58.5%)		
Unknown	22 (10.8%)		

2) Socioeconomic status: associated factors within the study population

Table 4 shows the univariate analysis of the association between SES and possible associated factors within men with KS.

Level of Education

There was a positive association between scores for subjective general health and level of education. There was no evidence for an association between age at diagnosis, presence of health problems, experienced discrimination, testosterone substitution or social activity and level of education.

*Occupational status*A positive association was found for subjective general health and occupation. Men with a (very) bad subjective general health were significantly more often without occupation, compared to men with (very) good subjective general health. Negative associations were found for the presence of health problems and occupation, and for experienced discrimination and occupation. A positive association was found between age of diagnosis and occupation. Men with KS who received their diagnosis in adulthood had significantly higher levels in occupation. There was no association found for testosterone substitution, social activity or occupation.

Satisfaction with income

A positive association was found for subjective general health and satisfaction with household income. Men who got their diagnosis at adulthood were significantly more satisfied with their household income compared to men who got their diagnosis of KS at childhood or adolescence. Furthermore, a positive association was found for subjective general health and satisfaction with household income. Men with a better subjective general health were more satisfied with their household income compared to those with a worse subjective general health. Finally, a negative association was found for experienced discrimination and satisfaction with household income. Men with KS who had experienced discrimination were significantly less satisfied with their household income compared to those who did not experience discrimination. There were no other significant associations for presence of health problems, testosterone substitution or social activity and satisfaction with household income.

Table 4 Linear regression analysis of the main outcomes and possibly associated factors in our study population of men with Klinefelter syndrome

	Independent variable	**Regression coefficient** (B)	**95% Confidence interval**	**P-Value**
Level of Education (Scale: 1-3)	Age at Diagnosis	0.120	-0.133; 0.372	0.350
	Subjective general health	**0.204**	**0.098; 0.310**	**<0.001**
	Presence of health problems	-1.106	-0.321; 0.110	0.334
	Experienced discrimination	-0.013	-0.242; 0.216	0.908
	Social activity	0.019	-0.084; 0.121	0.721
	Testosterone substitution	0.209	-0.312; 0.729	0.429
Current Occupation (Scale: 1-2)	**Age at Diagnosis**	**0.183**	**0.007; 0.359**	**0.041**
	Subjective general health	**0.155**	**0.081; 0.229**	**<0.001**
	Presence of health problems	**0.218**	**0.372; 0.065**	**0.006**
	Experienced discrimination	**-0.307**	**-0.471; -0.144**	**<0.001**
	Social activity	0.027	-0.048; 0.102	0.476
	Testosterone substitution	0.120	-0.230; 0.470	0.499
Satisfaction with income (Scale: 1-4)	Age at Diagnosis	-0.277	-0.585; 0.031	0.077
	Subjective general health	**-0.199**	**-0.334; -0.065**	**0.004**
	Presence of health problems	0.209	-0.068; 0.486	0.139
	Experienced discrimination	**0.435**	**0.138; 0.731**	**0.004**
	Social activity	0.077	-0.053; 0.207	0.242
	Testosterone substitution	0.209	-0.496; 0,913	0.559

3) Latent structure analysis

Based on the subgroup of men with KS of 25 years and older, a structural equation model was built to explore the relationships between all variables mentioned above, except for testosterone substitution, experienced discrimination, and age of KS diagnosis. Testosterone substitution was removed from the model as it was frequently missing (51/205 missing) and almost always positive if non-missing (145/154). The role of “experienced discrimination” in the SEM was unclear, for example, it was unclear whether it was a cause or an effect in the relation with occupation. Therefore, it was not included in the model. Inclusion of age of diagnosis resulted in worse model fit and it was not included in the final model. Possibly this variable was too much affected by current age of the participants and country to result in clear patterns. Due to missing data, data of 137/164 men with KS ≥ 25 years were included in the final SEM.

The resulting exploratory model (see Figure 2) shows the relationships between all other variables mentioned above. Overall, it indicates a strong impact of the severity of KS and the presence of health problems on the social economic status outcomes occupation and satisfaction with income. Lower levels of education were associated with a worse subjective general health. A better general health was associated with higher levels of occupation. Having an occupation was related to higher levels of satisfaction with income.(see Table 5).

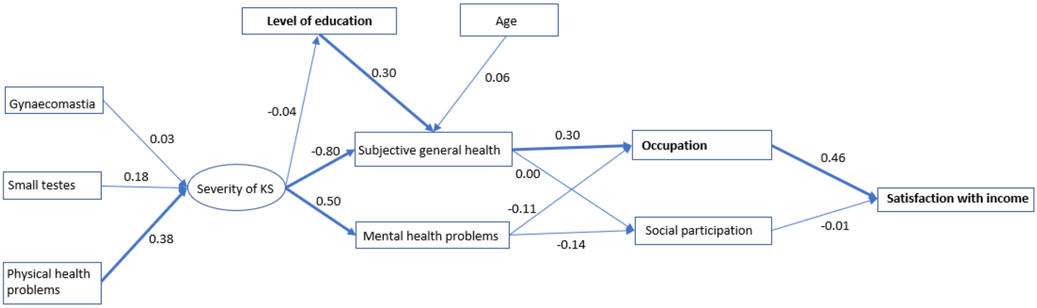

Figure 2 Structural equation model to explore relations between health variables, age, social participation, and social economic status in men with KS. The variable “Severity of KS” was calculated based on gynaecomastia, small testes, and physical health problems. Statistically significant relationships are shown with a fat printed arrow. The SEM model fit criteria were satisfactory: Chi-square was 31.08 with 24 degrees of freedom (p-value 0.15), the root mean square error of approximation (RMSEA) was 0.0466, and the Bentler Comparative Fit Index (CFI) was 0.9378.

Table 5 Impact of the severity of KS on different variables, using a structural equation model (SEM). Presented are the standardized total effects (i.e. sum of direct and indirect effects).

	Correlation coefficient	**Standard error (SE)**	**P-Value**
Education	**-0.04**	**0.14**	**0.80**
Current occupation	**-0.30**	**0.07**	**<0.01**
Satisfaction with income	**-0.14**	**0.04**	**<0.01**
Social participation	-0.07	0.07	0.35
Mental health problems	0.50	0.11	<0.01
Physical health problems	0.38	0.10	<0.01
Subjective general health	-0.80	0.15	<0.01

The SEM model fit criteria were satisfactory: Chi-square was 31.08 with 24 degrees of freedom (p-value 0.15), the root mean square error of approximation (RMSEA) was 0.0466, and the Bentler Comparative Fit Index (CFI) was 0.9378.

Discussion

This is the first European multicenter study comparing education and socioeconomic outcomes of 218 men with KS with data from a European reference population of more than 1500 men. Our study shows that men with KS achieve lower levels of education and occupation and report less satisfaction with income compared to the ESS reference population.

Level of Education

Our observation that fewer men with KS achieve a high level of education compared to the ESS reference population is in accordance with an earlier Danish registry study [8]. They compared socioeconomic parameters in 1049 men with KS with 100,824 men of the general Danish population [8]. Possible contributing factors to lower levels of education in men with KS may be a delay in neurocognitive development and a higher presence of both mental and somatic comorbidity [7,15]. This is supported by the results of our univariate analysis, showing an association between lower scores for general health and lower levels of education and our SEM analysis, that revealed a significant impact of level of education on subjective general health. Numerous studies report on learning problems, especially related to language [11,16,17] and difficulties in executive function in KS [18]. Males with KS who are diagnosed during childhood have been reported to have a more severe phenotype, compared to men diagnosed at adulthood mainly due to fertility problems [19]. It can be speculated that early diagnosis and subsequent individualized interventions may have a positive effect on level of education, counteracting the effect of a more severe phenotype, especially on learning difficulties [20]. We did not find an association between testosterone therapy and the level of education. This could be due to the fact that almost all men with KS in our study have had testosterone supplementation. However, earlier studies reported no improvement of cognitive performance in patients with KS after testosterone supplementation [21,29]. In contrast, one study suggested that insufficient hormonal substitution may contribute to an increase in psychosocial morbidity, defined as an impaired ability to function based on the combined influence of psychological factors and the surrounding social environment [22]. Furthermore, a prospective study by Samango-Sprouse et al. has found that early testosterone supplementation for 29 children with KS compared to no testosterone supplementation (57 children in the control group) had a significant benefit for social communication and social cognition [26]. For clarification, a large randomized controlled study with long term follow up would be useful to investigate potential benefits or harms of testosterone therapy on SES in adolescents with KS.

Occupational status

In our study, the risk of having no occupation was increased for men with KS compared to the ESS reference population. Reasons for no occupation were a higher risk for being unemployed, actively looking for a new job; being permanently sick or disabled; being in retirement. Our findings are supported by a Danish registry study investigating men with KS, reporting a hazard ratio (HR) of 2.4 (2.1 – 2.4) for early retirement, compared to a matched control group [8]. Accordingly, another study found the median age of retirement to be significantly lower for men with KS compared to a matched control group (43 years vs 60 years) [27]. Skakkebaek et al. found a higher incidence of absence from work due to illness amongst men with KS (51%) compared to a matched control group (32%). Furthermore, they also reported a significantly higher percentage of men with KS to be retired compared to the age-matched control group [28]. A possible explanation for higher levels of having no occupation of men with KS was given by our latent structure analysis. A higher severity of KS, a worse general health status and the presence of health problems led to a lower occupational status of men with KS. Therefore, more emphasis should be given to establish and evaluate individualized support programs to increase general health of men with KS.

Satisfaction with income

The present study showed a lower satisfaction rate with current household's income amongst men with KS when compared to the ESS reference population, confirming the results of a national Danish cohort study [8,23] and a more recent study about socioeconomic status in men with KS [28]. We were able to show in our latent structure analysis that a higher severity of KS and lower levels of subjective general health led to lower levels of occupation and subsequently to lower levels of satisfaction with income. The treatment of men with KS may be improved by addressing KS-related physical and mental health problems [28].

Limitations

A main limitation of this study is the possibility of selection bias, as men with KS were mostly recruited from participating outpatient clinics and from patient support groups (for more information see Röhle et al., 2017, [12]). Unfortunately, it is unknown how many possible participants have been contacted at the different recruiting clinics and patient support groups. Furthermore, the questionnaire used in this study was rather long, taking about 3 hours to fill in, which might have led to a selection bias in favor of the cognitively better functioning men with KS. However, there was less than 10% missing data for all questions, except for the question about satisfaction with income, which 15% of men with KS did not answer.

Additionally, the long questionnaire might have led to a relatively low number of included men with KS. Another limitation of this study is its retrospective, explorative design and that parts of the questionnaire contained self-constructed questions which contained subjective outcomes and were not validated. In the linear regression analysis, there was no adjustment for multiple comparisons because of the exploratory nature of this study and the primary concern about type II error. The results of the latent structure analysis should ideally have been based on more participants, and therefore the results should be considered exploratory.

Conclusion

Men with KS achieve on average lower levels of education, occupation and report less satisfaction with income compared to the ESS reference population. The presence of health problems and lower scores of general health were associated with lower levels of occupation and lower satisfaction with income in men with KS. Further studies are necessary to evaluate the effect of an earlier diagnosis and individualized support programs.

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Availability of data and materials The datasets analyzed during the current study are not publicly available as long as primary analyses for other outcomes of dsd-LIFE are not completed. Afterwards scientific public use files are planned. The data will be made available by the principal investigator upon request to researchers after publication of the primary outcomes described in the grant by the consortium.

Disclosure summary

All authors declare no support from any organization for the submitted work; no relationship with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work. Therefore, the authors declare that they have no competing interests.

Trial registration: German Clinical Trials Register: Registration identification number: DRKS00006072, date of registration April 17th, 2014.

DRKS00006072 (German Clinical Trials Register).

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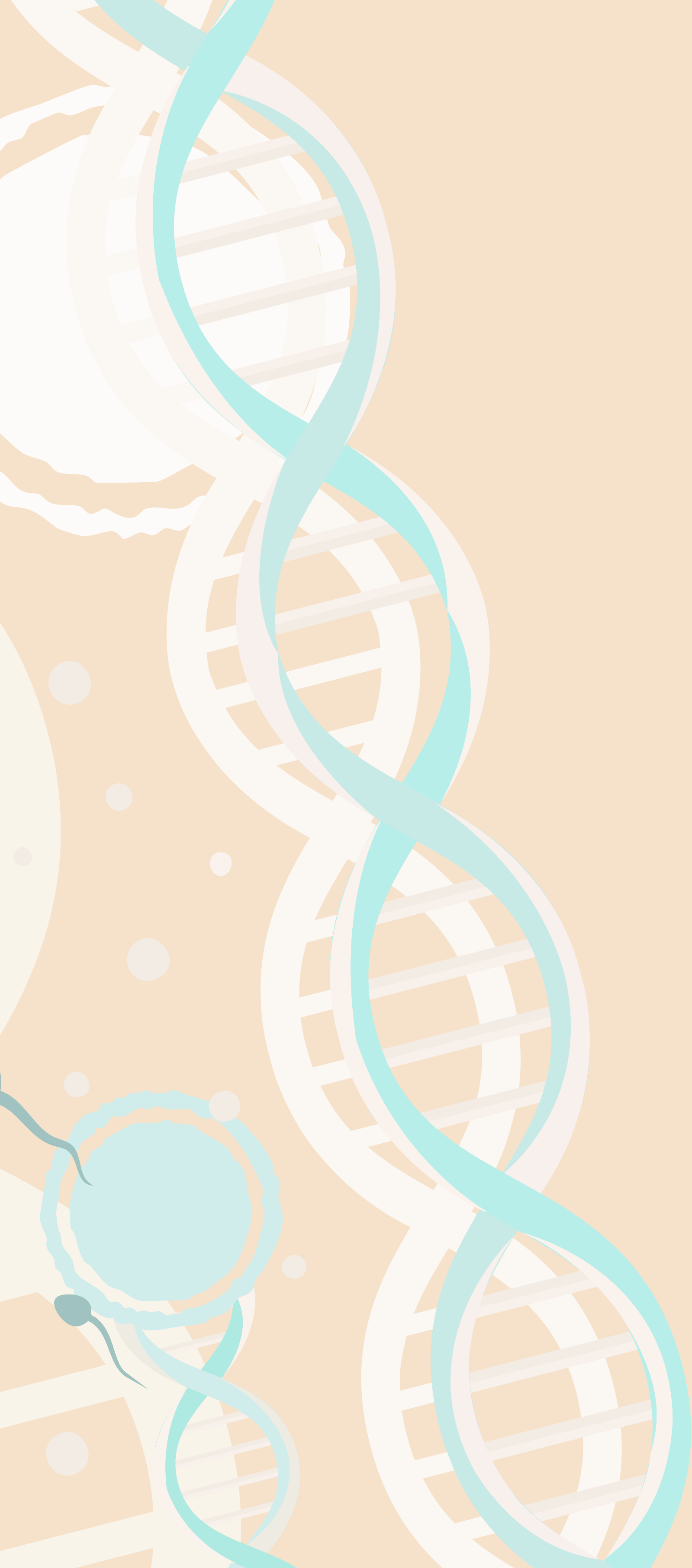

Chapter VII

Discussion

Integrating Findings with Current Literature

This thesis aimed to provide a comprehensive analysis of Klinefelter Syndrome (KS), focusing on its impact on spermatogenesis and fertility, quality of life (QOL), socioeconomic status (SES), and healthcare provision. The findings from the five chapters are discussed below in the context of the current literature, emphasizing the advancements and the ongoing challenges in understanding and managing KS.

Fertility Preservation in KS

With our comprehensive review on fertility preservation in KS, we have synthesized the current evidence and produced several key findings that have important implications for clinical practice and future research.

In our analysis, we found that the reproductive potential of pre-pubertal children with KS is already compromised, though the exact mechanisms remain unclear. The degeneration of germ cells in KS appears to start early, possibly even in fetal life, with studies showing reduced numbers of germ cells in testicular biopsies from KS fetuses and infants [1,2]. However, gonadotropine levels during childhood are generally normal in boys with KS [2,3]. A key finding that we describe in our review is that major histological changes in the testes occur during puberty. There is an initial proliferation of Sertoli and interstitial cells, followed by an accelerating decline of germ cells, hyalinization of seminiferous tubules, and degeneration of Sertoli cells [2]. This progressive degeneration results in shrinking of the testes to a prepubertal size of 2-4 ml [4]. Regarding fertility preservation options, we found that spermatozoa are extremely rare in the ejaculates of adolescents with KS. Across multiple studies we reviewed, only 1 out of 62 adolescents (1.6%) had spermatozoa in their ejaculate [5-8]. This indicates that sperm cryopreservation from ejaculates is not a viable fertility preservation option for the vast majority of adolescents with KS.

For testicular sperm extraction (TESE), we found that the success rates vary considerably based on the age of KS patients, which is a crucial point of our review. In adolescents younger than 16 years old, sperm retrieval rates by TESE were very low, ranging from 0-20% [2,5]. In contrast, for KS patients between 16-30 years old, sperm retrieval rates were much higher at 40-70% [7,9]. This difference in retrieval rates based on age is a key consideration for the timing of fertility preservation attempts. Importantly, our analysis showed that while spermatogonia can be found by TESE in about 50% of peri-pubertal adolescents with KS, there are currently no clinically functional techniques to use these immature germ cells for fertility preservation [5,10]. However, the creation of spermatozoa from embryonal stemcells, iPS cells, and SSCs is theoretically an option and a topic of research, further developments of this technology are expected in the near future [11].

In adult men with KS, we found that about 8% are able to ejaculate spermatozoa [12-14]. For those who cannot, TESE has been shown to be successful in retrieving sperm in about 50% of cases [15]. This relatively high success rate in adults, despite severe testicular degeneration, is an intriguing finding that warrants further investigation into the mechanisms of residual spermatogenesis in KS.

Another crucial aspect of our review was examining potential predictive factors for successful sperm retrieval. Contrary to some previous assumptions, we found that most factors such as hormones (FSH, LH, testosterone, inhibin B), testicular volume, and testicular histology have not shown reliable predictive value for sperm retrieval rates [16-18]. One study suggested that a combination of total serum testosterone above 7.5 nmol/l and LH levels below 17.5 U/l may predict higher retrieval rates [7].

Regarding the impact of testosterone treatment on fertility preservation, our analysis revealed that, contrary to some earlier concerns, testosterone treatment does not appear to have a significant negative impact on future TESE outcomes in KS patients [9,19]. This is an important finding for clinical management, as it suggests that clinically indicated androgen treatment should not be withheld from adolescents with KS due to fertility concerns. This reflects the recommendation from the current andrology guideline on management of KS [20].

Based on our comprehensive review of the current evidence, we have concluded that fertility preservation should not be offered to children and adolescents with KS younger than 18 years old, except in the context of approved research protocols. The low sperm retrieval rates in this age group, combined with the lack of techniques to use immature germ cells, do not justify the potential risks and psychological impact of the procedure. Instead, we recommend that TESE for fertility preservation in KS patients is best performed between the ages of 18 and 30. This recommendation is based on the higher sperm retrieval rates observed in this age range, balanced against the potential decline in success rates seen in some studies for men over 35 [17,21]. A recent prospective study including 119 adolescents and adults with KS supports our findings, as the sperm retrieval rate by TESE was similar in the young (15-22y) and older (23-45y) group [22].

Implications for Practice:

1. Testosterone treatment, when clinically indicated, should not be withheld from adolescents with KS due to fertility concerns.
2. Clinicians should counsel KS patients and their families that early fertility preservation (before age 18) cannot guarantee future fertility and may even reduce chances for offspring by removing functional immature germ cells.
3. Sperm cryopreservation from ejaculates should be attempted in adult KS patients before considering TESE, given that about 8% may have sperm in their ejaculate.
4. Regular follow-up of KS patients should include discussions about fertility preservation options as they enter the optimal age range for TESE.
5. TESE for fertility preservation should be considered for KS patients between ages 18-30, with full counseling about success rates and alternatives.

Implications for Future Research:

1. Longitudinal studies tracking germ cell development and hormonal changes from childhood through adulthood in KS patients are needed to better understand the progression of testicular failure.
2. Further research is required to develop and validate predictive markers, such as gene panels or epigenetic modifications, for successful sperm retrieval in KS patients.
3. Investigation into the mechanisms of residual spermatogenesis in adult KS patients could provide insights for developing new fertility preservation strategies.
4. Studies on the psychological impact of fertility preservation procedures on adolescents with KS are needed to improve ethical guidelines and counseling practices.
5. Research into techniques for maturing and using immature germ cells (spermatogonia) for fertility purposes could potentially expand options for younger KS patients in the future.
6. Large, multi-center studies are needed to definitively determine the optimal age range for TESE in KS patients and to clarify any potential negative effects of delaying the procedure.

In conclusion, our comprehensive review of fertility preservation in KS has revealed that while the condition presents significant challenges to fertility, there are viable options for many patients, particularly when timed appropriately. The current evidence strongly suggests that TESE for fertility preservation should be considered for KS patients between ages 18-30, but not earlier. Testosterone supplementation during puberty and adolescence does not impair future fertility. This work highlights the need for individualized counseling and management of KS patients regarding fertility preservation, as well as the importance of continued research to improve outcomes and expand options for these individuals.

Chromosomal Origins and Spermatogenesis in Klinefelter Syndrome

Our study explored whether the origin of the additional X-chromosome (maternal or paternal) in Klinefelter Syndrome (KS) could be a determinant of spermatogenesis and subsequent fertility outcomes. The study involved 35 non-mosaic KS patients undergoing testicular sperm extraction (TESE) to evaluate the presence of spermatozoa. Despite hypothesizing that the parental origin of the extra X-chromosome might influence spermatogenesis, our findings revealed no significant correlation, challenging assumptions about the genetic mechanisms underpinning spermatogenesis in KS.

Our findings align with the broader literature indicating that spermatogenesis in KS is influenced by complex genetic and epigenetic factors rather than the parental origin of the additional X-chromosome. Previous studies have documented an incidence of sperm retrieval in KS typically less than 50%, with significant variability across individuals [13,20]. The lack of correlation between X-chromosome origin and spermatogenesis observed in our study further supports the hypothesis that additional genetic or environmental factors, possibly including gene dosage effects or the presence of specific X-linked genes, contribute to the variability in fertility outcomes among KS patients. A recent study has confirmed that skewed X chromosome inactivation in men with KS is comparable to skewed X chromosome inactivation in women [23]. More extensive and unbiased studies would be required to conclusively establish the influence of both the parental origin of the extra X chromosome and the impact of skewing in X inactivation on the Klinefelter syndrome phenotype. The same applies for DNA methylation and RNA expression. A recent study reported that as the number of X chromosomes increases, the active X chromosome undergoes organizational changes, decreasing in size and compacting its chromatin [24]. These findings align with previous research demonstrating a similar chromatin conformation pattern for the inactive X chromosome in both Klinefelter syndrome patients and biological 46,XX females [25]. Another study discovered a subpopulation of Sertoli cells in Klinefelter syndrome individuals that lack X-inactive specific transcript (XIST) expression, leading to increased X-linked gene expression in this cell group [26]. Through pathway analysis, the authors identified numerous signaling pathways that appear to be upregulated in Klinefelter syndrome. Overall, the findings support a model where pathogenic changes in the testicular interstitium originate from the loss of X inactivation in pubertal Sertoli cells independent from maternal or paternal origin, and suggest that dysregulated secreted factors from Sertoli cells may contribute to the Klinefelter phenotype [26]. The potential biological implications of these modifications observed in KS warrant further investigation.

Up to date, the only possible positive predictors of spermatogenesis in men with KS are a combination of low LH <17.5U/L and high testosterone levels >7.5nmol/L or the presence of higher AMH levels [7]. Unfortunately, no specific cut-off level was presented, so further research is necessary [22].

Implications for practice:

1. Counseling: Clinicians should be aware that the origin of the additional X-chromosome does not influence their chances of successful sperm retrieval. Fertility counseling should focus on other factors, including hormonal assessments and histological evaluations, while acknowledging the inherent unpredictability of TESE outcomes in KS.
2. Individualized Care: Given the variability in spermatogenic potential among KS patients, a personalized approach to fertility preservation should be adopted. This may include repeat TESE attempts or exploring emerging fertility preservation techniques.
3. Research-based Protocols: In the absence of reliable genetic markers, fertility preservation in KS should be carefully considered, and only done within research protocols to ensure ethical and evidence-based practices. A European registry could help to improve standards and accelerate research.

Implications for future research:

1. Genetic mechanisms: Further research is required to elucidate the genetic and epigenetic mechanisms that influence spermatogenesis in KS. Large-scale genomic studies may help to identify specific X-linked genes or regulatory elements that affect spermatogenesis.
2. Predictive markers: There is an urgent need to develop and validate reliable biomarkers for predicting TESE outcomes. Markers such as LH/FSH ratio, AMH or maybe gene expression panels in the future could significantly enhance clinical decision-making and patient counseling.
3. Technological innovations: Research into advanced reproductive technologies, including in vitro maturation of spermatogonia, holds promise for expanding fertility options for KS patients, particularly those with low or no sperm retrieval via TESE.

The findings from our study suggest that the origin of the additional X-chromosome does not serve as a predictive marker for spermatogenesis in KS. This underscores the complexity of genetic influences on fertility in KS and highlights the need for continued research into more reliable predictive factors.

Quality of Healthcare

Chapter IV evaluated the quality of healthcare received by KS patients. This chapter provides crucial insights into the current state of healthcare provision for KS patients, highlighting both strengths and areas for improvement. The findings underscore the importance of specialized, multidisciplinary care in managing this complex condition.

Our studies showed that many KS patients are generally moderately satisfied with their healthcare, but significant gaps remain. Some patients reported that their concerns were not taken seriously by healthcare providers with a lack of understanding the unique needs of KS, such as sexual problems as a result of low testosterone, infertility and psychological problems. These findings are consistent with other studies, highlighting similar issues in patient satisfaction among KS populations [27,28]. The discrepancy between general satisfaction and these specific concerns suggests that while overall care may be adequate, there are particular aspects of KS management that require more attention and specialized knowledge from healthcare providers. The heterogeneity in the presentation of KS may be a complicating factor in providing adequate healthcare and underscores the need for an individualized patient-centered approach. The varying ages at diagnosis, with many individuals diagnosed during adulthood, is a recurring theme in the literature [29,30]. In our survey on the satisfaction with medical support received during childhood and adolescence, a significant finding was that 50.3% of the respondents did not provide a response as they had not received any support during this early period. This lack of early medical intervention is noteworthy, as it may have implications for the timely diagnosis and management of KS. Delay in the diagnosis of KS was reported in another study, emphasizing the need for improved awareness among healthcare professionals and the development of specialized care pathways for timely diagnosis and management [31]. Early diagnosis and intervention could improve long-term outcomes for KS patients, making this an important area for improvement in KS healthcare.

Our study showed that satisfaction with healthcare among men with KS is moderate and influenced by different factors, with accessibility of healthcare and general health scores being the most important indicators. A majority of participants

(65.4%) reported having a regular general physician (GP) who attends to their health needs. However, it is important to address that 1/3 reported not to visit a GP regularly over the past 12 months. A majority of 66.8% reported seeing one or more specialists trained in their condition. A substantial proportion of participants reported finding it difficult to find a specialist trained with their condition. Similar challenges were observed in scheduling appointments, although the majority reported it was not difficult to secure an appointment. These findings highlight the need for comprehensive and multidisciplinary care to address the diverse medical and psychosocial needs of individuals with KS and the importance of improving access to specialized care and reducing barriers in finding and obtaining appointments, ensuring timely and appropriate support for individuals with KS [32].

Developing and implementing integrated care models can address the multifaceted needs of KS patients. These models involve a multidisciplinary team of healthcare providers, including a well-informed GP that coordinates endocrinologists, geneticists, psychologists, and social workers, working together to provide comprehensive care. Integrated care models improve coordination among providers, ensure all aspects of KS are addressed, and enhance overall patient satisfaction and outcomes.

The importance of multidisciplinary care is supported by studies, which highlight the positive impact of comprehensive care on healthcare outcomes and behavioral phenotypes in individuals with KS [31,33].

Educating and training healthcare providers about KS is essential for improving the quality of care. GPs need to be aware of the specific symptoms, challenges, and needs of KS patients, as they have a higher risk for many morbidities [34]. Continuing medical education programs and training workshops can equip providers with the knowledge and skills necessary to deliver high-quality care to KS patients such as screenings for metabolic syndrome, psychological support or fertility counseling. The reported difficulties in finding specialists trained in KS underscore the need for more widespread education about the condition among healthcare providers. This is particularly important given the wide range of symptoms and comorbidities associated with KS, which require a broad understanding across multiple medical specialties. Consistent with Herlihy et al. (2018), the current study suggests potential gaps in preventive healthcare measures for individuals with KS [35]. Lower rates of cancer screening and vaccinations among individuals with KS indicate the need for targeted interventions to ensure appropriate preventive care in this population. This highlights the importance of healthcare providers being proactive in addressing the specific healthcare needs of individuals with KS.

Implications for Future Research and Practice

1. Develop and implement standardized care protocols and guidelines specific for children, adolescents and adults with KS, ensuring comprehensive and coordinated care across multiple specialties allowing early discovery of comorbidities.
2. Enhance education and training programs for healthcare providers to improve their understanding of KS and its management.
3. Improve access to specialized care, particularly in areas such as endocrinology, reproductive health, and mental health.
4. Implement screening programs for early diagnosis of KS. This could be established by non-invasive prenatal diagnostic testing during pregnancy or in pediatric populations by screening for small testicles.
5. Develop patient education programs to empower KS patients with knowledge about their condition and available healthcare resources.
6. Conduct further research into the reasons for healthcare underutilization among KS patients and develop strategies to address these barriers.
7. Evaluate and enhance preventive care measures for KS patients, including appropriate screenings e.g. for metabolic diseases and cancer.

In conclusion, Chapter IV of this thesis provides valuable insights into the current state of healthcare for KS patients. While there are areas of satisfaction, significant gaps remain, particularly in access to specialized care and early diagnosis and support. The findings underscore the need for a multidisciplinary, integrated approach to KS care, with a focus on improving provider education, enhancing access to specialized services, and implementing standardized care pathways. By addressing these areas, there is potential to significantly improve the quality of healthcare and overall outcomes for individuals with KS.

Quality of Life

In Chapter V we describe the quality of life (QoL) in KS patients, revealing lower QoL compared to the general population. This chapter provides crucial insights into the multifaceted impact of KS on various aspects of patients' lives, highlighting the need for comprehensive interventions to improve overall well-being.

Our study has shown that global QoL in men with KS is significantly lower compared to a UK reference population. This finding is particularly important as it quantifies the extent of QoL impairment in KS patients and provides a basis for comparison with other populations. Interestingly, the global QoL is lowest amongst those persons with KS who had experienced discrimination during their life or suffer from chronic mental health problems. This highlights the negative impact of discrimination in general on the overall well-being. Further research should investigate, if men with KS are more susceptible for discrimination compared to the general population. The importance of health problems is supported by a previous, smaller study investigating QoL in 43 adolescents with KS, reporting that a poor outcome in QoL directly correlated to the severity of the phenotype [36]. The severity of the phenotype was measured as a composite score of physical traits including tall stature, eunuchoid body proportion, wide arm span, large waist circumference, high BMI, small testicular volume, short phallus, or gynecomastia. This suggests that the physical manifestations of KS play a significant role in determining QoL. Furthermore, lower scores compared to a reference population for QoL, self-esteem, body image, and mental health were reported in a study using validated questionnaires like the "Personal Wellbeing Index" and the "Rosenberg Self-esteem Scale" among 87 adult men with KS [37]. This aligns with our findings and emphasizes the multidimensional nature of QoL impairment in KS. The findings of this chapter are consistent with numerous studies reporting reduced QoL due to physical, cognitive, and psychosocial challenges [38,39]. Sexual dysfunction, depression, and anxiety were identified as significant predictors of reduced QoL in KS patients, aligning with previous research [40,41]. The clinical symptoms of KS, such as hypogonadism, gynecomastia, and reduced muscle mass, contribute significantly to lower QoL. These physical manifestations can affect body image, self-esteem, and overall physical well-being. Cognitive impairments, including difficulties with language, attention, and executive function, exacerbate these challenges [42]. These cognitive issues can impact educational attainment, occupational performance, and social interactions, further diminishing QoL [42]. A comprehensive management approach addressing physical, psychological, and social aspects is crucial for improving the overall well-being and QoL of KS patients, as suggested by recent reviews and clinical guidelines [20,43,44].

In our study, there was no significant association between age of diagnosis and QoL. This is an interesting finding, as it contrasts with the general assumption that earlier diagnosis leads to better outcomes. It's possible that the impact of early diagnosis on QoL is mediated through other factors, such as earlier access to treatment and support. There was also no significant association between QoL and testosterone supplementation at present. However, these results should be interpreted with caution due to the distribution of participants receiving testosterone supplementation. Of 205 participants, 145 had testosterone supplementation, only 9 did not, and for the remaining participants, it was unknown. This finding was confirmed by another study investigating QoL in 132 men with KS in Denmark, which also found no significant difference between the two KS subgroups with or without testosterone therapy [45]. Compared to a European reference population with a diagnosis of various psychiatric illnesses such as depression and schizophrenia, the scores were higher for men with KS in the physical, psychological and environmental QoL domains but not in the social domain. This finding confirms earlier studies indicating that participation in social activities often remains challenging for men with KS [46,47].

The lower QoL in the psychological health domain may be explained by the finding that depression and anxiety are often present in men with KS [47]. Potentially contributing factors such as bullying, lower self-esteem issues and social challenges should be carefully evaluated by healthcare providers. The psychosocial impact of KS affects interpersonal relationships, social integration, and self-esteem. KS patients are at higher risk for mental health issues, including depression, anxiety, and social isolation [48].

A novel finding of this study is that men with KS reported less participation in social activities compared to the healthy European reference population and reported lower scores in the quality of life social domain. Their overall quality of life social domain scores were even lower when discrimination based on condition was experienced. Several studies reported higher levels of distress during social interactions, shyness and social anxiety and withdrawal amongst men with KS [45,49,50]. The social challenges faced by KS patients can be particularly detrimental to QoL. Difficulties in social interactions, coupled with potential stigma associated with the condition, can lead to social withdrawal and isolation. This social dimension of KS highlights the need for interventions that promote social skills development and community integration. A recent small study has investigated the effectiveness of a social management training in 16 adolescents, showing promising results [51]. The need for early social support is further emphasized by a study showing that employment status and social support are amongst the best predictors of psychosocial well-being [47]. These findings underscore the importance of psychological support and interventions to improve mental health outcomes in KS patients.

Implications for Future Research and Practice

1. Develop comprehensive, multidisciplinary interventions that address physical, cognitive, and psychosocial aspects of KS. A well-trained GP could coordinate further support by endocrinologists and psychologists for example.
2. Implement early screening and intervention programs to address cognitive and social skills deficits. A well trained psychologist could support multidisciplinary care teams for men with KS. Special focus could be on depression and anxiety management.
3. Develop targeted interventions to improve sexual health and function in KS patients. Awareness for the necessity of testosterone supplementation and support from a sexologist should be evaluated.
4. Implement social skills training programs and support groups such as patients associations to enhance social integration and reduce isolation.
5. Conduct public awareness campaigns to reduce stigma and discrimination associated with KS.
6. Further investigate the relationship between testosterone therapy and QoL in large cohort studies.
7. Explore the potential benefits of early diagnosis and intervention on long-term QoL outcomes.

In conclusion, Chapter V of this thesis provides valuable insights into the complex impact of KS on quality of life. The findings highlight the multifaceted nature of QoL impairment in KS, encompassing physical, psychological, and social domains. The significant impact of discrimination and social challenges on QoL underscores the need for a holistic approach to KS management that goes beyond medical treatment to address social and psychological needs. By addressing these various aspects, there is potential to significantly improve the overall quality of life and well-being of individuals with KS.

Socioeconomic Status

Chapter VI revealed significant socioeconomic challenges faced by KS patients, including lower educational attainment, higher unemployment rates, and financial difficulties. These findings align with previous research indicating socioeconomic disadvantages compared to the general population [12,14].

Educational support is crucial for KS patients, who often face learning disabilities and academic challenges. Tailored educational interventions, such as individualized education plans (IEPs) and specialized tutoring, can help KS patients overcome these barriers [30,52].

Our observation that fewer men with KS achieve a high level of education compared to the ESS reference population is in accordance with an earlier Danish registry study. They compared socioeconomic parameters in 1049 men with KS with 100,824 men of the general Danish population [53]. Possible contributing factors to lower levels of education in men with KS may be a delay in neurocognitive development and a higher presence of both mental and somatic comorbidity [36,37]. This is supported by the results of our univariate analysis, showing an association between lower scores for general health and lower levels of education. Our structural equation modelling (SEM) analysis revealed a significant impact of level of education on subjective general health. Numerous studies report on learning problems, especially related to language and difficulties in executive function in KS [37,52]. Males with KS who are diagnosed during childhood have been reported to have a more severe phenotype compared to men diagnosed in adulthood mainly due to fertility [54,55]. It can be speculated that early diagnosis and subsequent individualized interventions may have a positive effect on the level of education, counteracting the effect of a more severe phenotype, especially on learning difficulties [56,57].

We did not find an association between testosterone therapy and the level of education. This could be due to the fact that almost all men with KS in our study have had testosterone supplementation. However, earlier studies reported no improvement of cognitive performance in patients with KS after testosterone supplementation [53]. In contrast, one study suggested that insufficient hormonal substitution may contribute to an increase in psychosocial morbidity, defined as an impaired ability to function based on the combined influence of psychological factors and the surrounding social environment [58]. Furthermore, another prospective study found that early testosterone supplementation in 29 children with KS compared to no testosterone supplementation (57 children in the control group) had a significant benefit for social communication and social cognition [56]. A larger randomized controlled study with long-term follow-up would be useful to investigate potential benefits or harms of testosterone therapy on SES in adolescents with KS.

In our study, the risk of having no occupation was increased in men with KS compared to the ESS reference population. Being asked about the lack of occupation, answers given were actively looking for a new job; being permanently sick or disabled; being in retirement. Our findings are supported by a Danish registry study investigating men with KS, reporting a hazard ratio (HR) of 2.4 for early retirement, compared to a matched control group [59]. Accordingly, another study found the median age of retirement to be significantly lower for men with KS compared to a matched control group [59]. Skakkebaek et al. found a higher incidence of absence from work due to illness amongst men with KS compared to a matched control group. Furthermore, they also reported a significantly higher percentage of men with KS to be retired compared to the age-matched control group [60]. A possible explanation for higher levels of having no occupation of men with KS was given by our latent structure analysis. A higher severity of KS, a worse general health status, and the presence of health problems led to a lower occupational status of men with KS. Therefore, more emphasis should be given to establishing and evaluating individualized support programs to increase the general health of men with KS. These programs could help KS patients develop the skills and qualifications needed to secure stable employment. Additionally, workplace accommodations and support ensure that KS patients can perform their job duties effectively, contributing to their financial stability and overall well-being.

Implications for Future Research and Practice

1. Longitudinal studies: Future research should focus on longitudinal studies starting already during childhood to understand the long-term impact of educational and employment support interventions on the socioeconomic status of KS patients.
2. Policy development: Policymakers and healthcare providers should collaborate to develop and implement targeted programs that address the specific educational and employment needs of KS patients, aiming to improve their socioeconomic status and overall quality of life.
3. Comprehensive support programs: Developing comprehensive support programs that integrate educational, vocational, and healthcare services can help KS patients overcome socioeconomic challenges and improve their quality of life.
4. Awareness and training: Increasing awareness and training among educators, employers, and healthcare providers about the unique challenges faced by KS patients can lead to better support and accommodations in educational and work environments.

In conclusion, KS patients face significant socioeconomic challenges, including lower educational attainment, higher unemployment rates, earlier retirement and financial difficulties. To effectively address these challenges, a multifaceted approach is essential. Targeted interventions, such as mentorship programs linking KS patients with professionals, can enhance job readiness. Additionally, establishing patient support groups can provide a crucial network for sharing experiences and resources, fostering a sense of community. Psychological support, including counseling and therapy, can help address mental health issues that may arise from social stigma and personal challenges, improving overall well-being.

Future research should explore the effectiveness of these support mechanisms on educational and employment outcomes for KS patients, aiming to create comprehensive systems that empower them to achieve their full potential and enhance their quality of life.

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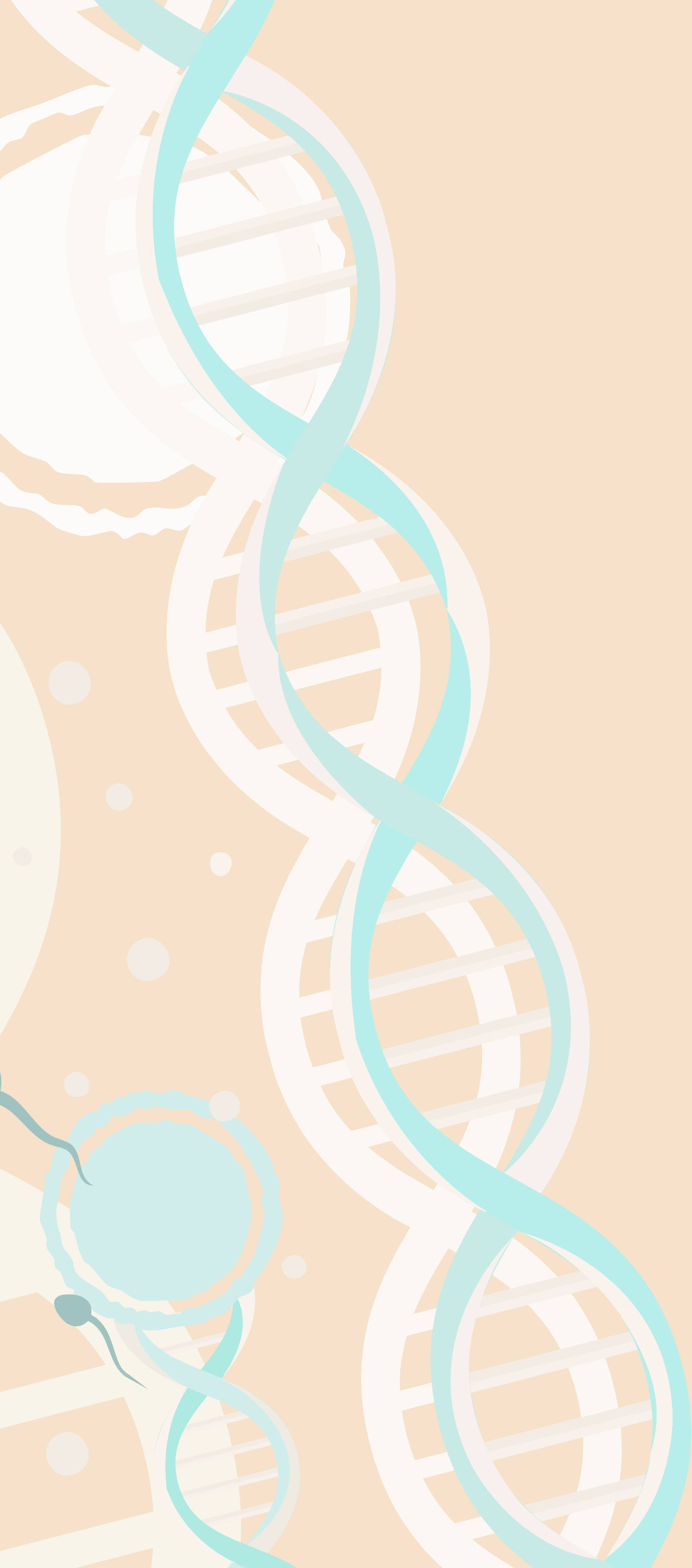


Chapter VIII

Summary

This thesis aimed to provide a comprehensive analysis of different aspects in Klinefelter Syndrome (KS), focusing on its impact on fertility, spermatogenesis, quality of life (QoL), socioeconomic status (SES), and healthcare provision. The findings from this thesis are summarized below, emphasizing the advancements and ongoing challenges in understanding and managing KS.

In **Chapter 1**, the general introduction, provides background information on KS. Epidemiology, (patho)physiology, spermatogenesis, fertility treatment, socio-economic status, quality of life and current healthcare approaches are discussed.

In **Chapter 2**, we give a comprehensive review of the literature about mechanisms of impairment of spermatogenesis in children and adolescents with KS and discuss options for fertility preservation. The current evidence does not support offering fertility preservation to pre-pubertal or pubertal children with KS due to absence of spermatozoa in the ejaculate and significantly lower retrieval rates of spermatozoa in TESE compared with adolescents (>18 years of age). Spermatogenesis in KS is characterized by a decrease in germ cells, increased apoptosis, and an altered ratio of germ cell types. Various sperm retrieval techniques, such as testicular sperm extraction (TESE) and microdissection TESE (micro-TESE), were discussed, with micro-TESE generally showing higher retrieval rates compared to conventional TESE. Age is crucial in determining the success of sperm retrieval, with significantly higher rates in young adults compared to pre-pubertal children. Therefore, the timing of TESE is critical, and fertility preservation should be considered in young adulthood.

In **Chapter 3**, we investigated whether paternal or maternal origin of the second X-chromosome was associated with spermatogenesis. Our results showed that neither maternal nor paternal origin of the extra X-chromosome can predict the presence or absence of spermatogenesis. This was the first study investigating X-chromosomal origin in combination with the presence of spermatozoa by TESE. Furthermore, hormone concentrations of LH, FSH und testosterone before TESE did not differ significantly between KS patients with our without spermatozoa.

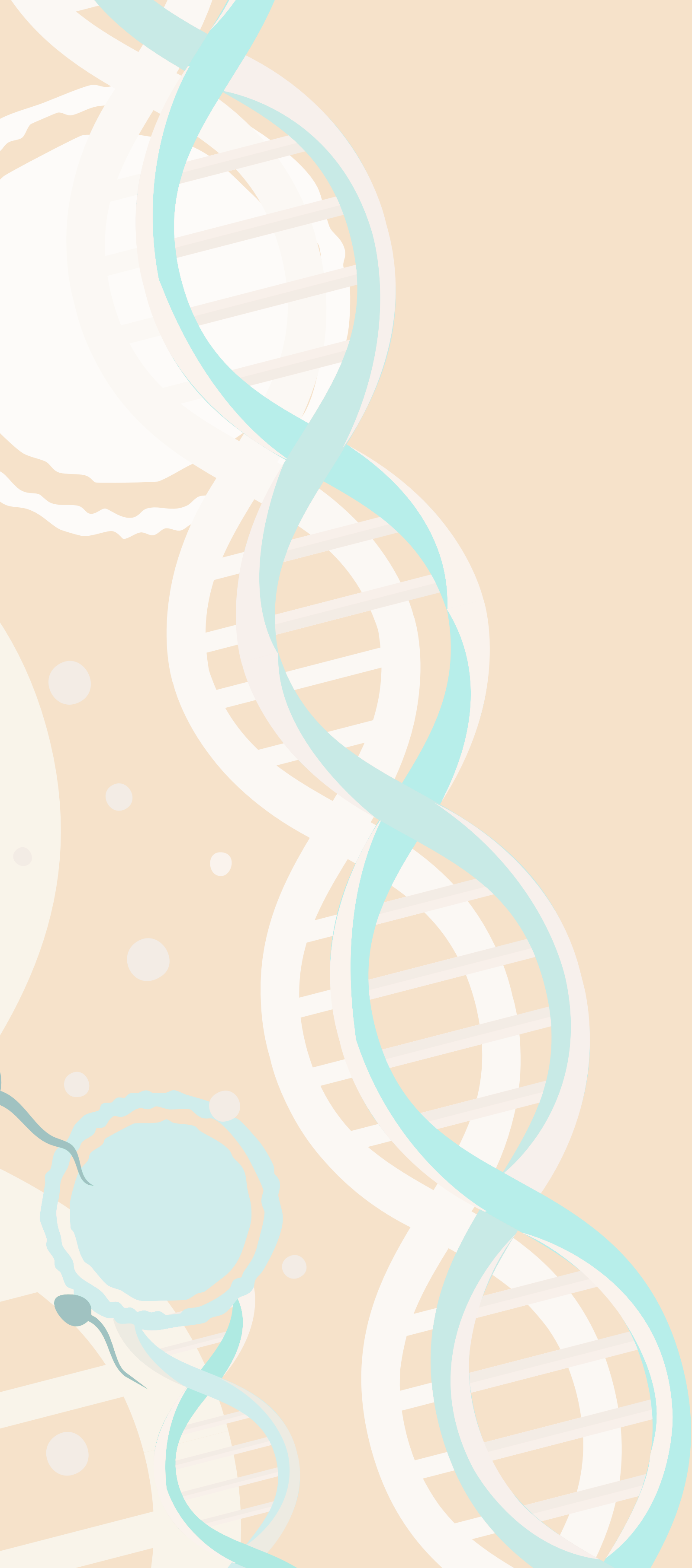
In **Chapter 4,** we show that general satisfaction with healthcare among men with KS is moderate and influenced by different factors, with accessibility of healthcare and general health scores being the most important indicators. A majority of participants (65.4%) reported visiting their GP regularly, and 66.8% reported seeing one or more specialists trained in their condition. Those findings may be not representative for the general population of men with KS, as all study participants have been recruited at highly specialized centers. While there are areas of satisfaction, significant gaps remain, particularly in access to specialized care and early diagnosis and support. The findings underscore the need for a multidisciplinary, integrated approach to KS care, with a focus on improving provider education, enhancing access to specialized services, and implementing standardized care pathways. By addressing these areas, there is potential to significantly improve the quality of healthcare and overall outcomes for individuals with KS.

In **Chapter 5**, we evaluate Quality of Life (QOL) using the WHO-QOL-BREF questionnaire. We revealed that global QoL in men with KS is significantly lower compared to a UK reference population. The global QoL is lowest amongst those persons with KS who had experienced discrimination during their life or suffer from chronic mental health problems. This highlights the negative impact of discrimination on the overall well-being of men with KS. Men with KS scored significantly lower for both physical and psychological QoL compared to a matched Danish cohort. This may be explained by the presence of chronic health problems and a higher risk for chronic diseases such as diabetes or heart diseases, which are also associated with lower scores for QoL.

A novel finding of this study is that men with KS reported less participation in social activities compared to the healthy European reference population and reported lower scores in the quality of life social domain. This may be due to higher levels of distress during social interactions, shyness, and social anxiety among men with KS.

In **Chapter 6**, we describe that men with KS achieve lower levels of education and occupation and report less satisfaction with income compared to the European Social Survey (ESS) reference population. Possible contributing factors to lower levels of education in men with KS may be a delay in neurocognitive development and a higher presence of both mental and somatic comorbidity. Our structural equation modeling (SEM) analysis revealed a significant impact of level of education on subjective general health. The risk of having no occupation was increased for men with KS compared to the ESS reference population. Reasons for a higher risk of being unemployed were being permanently sick or disabled, or being in retirement. Our latent structure analysis showed that a higher severity of KS, worse general health status, and the presence of health problems led to a lower occupational status of men with KS.

In **Chapter 7**, we discuss the research questions formulated at the end of the general introduction. Implications of the knowledge obtained from the studies performed for current clinical practice are discussed. Future research should focus on predictors of sperm retrieval by TESE in adult males with KS. Furthermore, a multidisciplinary approach should be implemented and evaluated, using integrated care models to face the highly variable yet enhanced risks for a broad diversity of physical and mental illness in men with KS. Many KS patients report gaps in healthcare access and provider knowledge. KS patients demonstrate significantly lower quality of life compared to the general population, with physical, psychological, and social factors contributing. KS patients also face substantial socioeconomic challenges, including lower educational attainment and higher unemployment, warranting targeted support programs. There is an up to date evidence based European Guideline for diagnostic and treatment of fertility in men with KS. However, such an interdisciplinary, evidence based and well-structured guideline is yet missing for the other areas of healthcare regarding KS, covering pubertal and cognitive development, advanced screening examinations and health support to ultimately improve QOL and SES in men with KS.



Chapter IX

Samenvatting

Dit proefschrift had als doel een uitgebreide analyse te bieden voor verschillende aspecten van het Klinefelter-syndroom (KS), met de nadruk op vruchtbaarheid, spermatogenese, kwaliteit van leven (QoL), sociaaleconomische status (SES) en de gezondheidszorgvoorziening. De bevindingen uit dit proefschrift worden hieronder samengevat. De vooruitgang bij het begrijpen en de lopende uitdagingen bij het beheren van KS worden benadrukt.

In **Hoofdstuk 1**, de algemene inleiding, wordt de epidemiologie, (patho)fysiologie, spermatogenese, vruchtbaarheidsbehandeling, sociaaleconomische status, levenskwaliteit van mensen met KS besproken en beschrijven wij de huidige organisatie in de gezondheidszorg.

In **Hoofdstuk 2** geven we een uitgebreid overzicht van de literatuur over de mechanismen, die betrokken zijn bij de spermatogenese bij kinderen en adolescenten met KS en bespreken we opties voor het behoud van de vruchtbaarheid. Met de huidige kennis wordt fertilitetispreservatie aan (pre-)puberale kinderen met KS vanwege significant lagere opbrengsten van spermatozoa niet ondersteunt. Spermatogenese bij KS wordt gekenmerkt door een afname van kiemcellen, toegenomen apoptose en een gewijzigde verhouding van kiemceltypen. Verschillende technieken voor het verkrijgen van zaadcellen, zoals testiculaire sperma-extractie (TESE) en microdissectie TESE (micro-TESE), werden besproken, waarbij micro-TESE over het algemeen hogere ophaalpercentages laat zien in vergelijking met conventionele TESE. Leeftijd is cruciaal bij het bepalen van het succes van het verkrijgen van zaadcellen waarbij significant hogere percentages worden waargenomen bij jongvolwassenen in vergelijking met (pre-)puberale kinderen. Daarom is de timing van TESE cruciaal en moet vruchtbaarheidsbehoud na de puberteit worden overwogen.

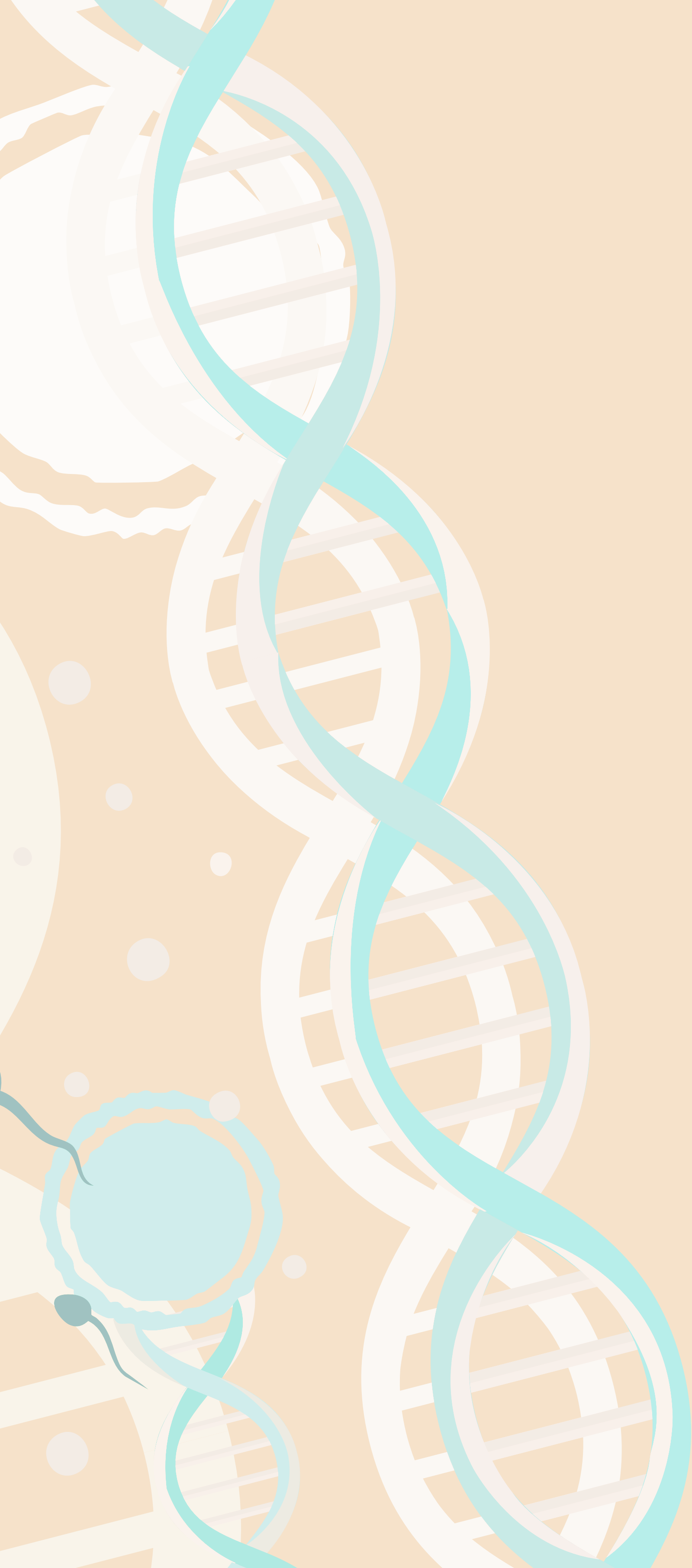
In **Hoofdstuk 3** hebben we onderzocht of de vaderlijke of moederlijke oorsprong van het tweede X-chromosoom geassocieerd is met de aanwezigheid van spermatogenese. Onze resultaten toonden aan dat noch de moederlijke noch de vaderlijke oorsprong van het extra X-chromosoom de aanwezigheid of afwezigheid van spermatogenese kan voorspellen. Dit is de eerste studie die de oorsprong van het X-chromosoom in combinatie met de aanwezigheid van spermatozoa door middel van TESE onderzocht.

In **Hoofdstuk 4** laten we zien dat mannen met KS matig tevreden zijn over de zorg die ze ontvangen. Deze slechte zorg wordt beïnvloed door verschillende factoren, waarbij de toegankelijkheid van de gezondheidszorg en de subjectief gevoelde gezondheid de belangrijkste factoren zijn. Een meerderheid van de deelnemers in onze studie (65,4%) gaf aan een vaste huisarts te hebben, en 66,8% meldde een of meer specialisten te zien die getraind waren in hun aandoening. Hoewel er op deelaspecten tevredenheid gerapporteerd is, blijven er aanzienlijke lacunes, vooral op het gebied van toegang tot gespecialiseerde zorg en vroege diagnose en ondersteuning. De bevindingen benadrukken de noodzaak van een multidisciplinaire, geïntegreerde benadering van KS-zorg, met de nadruk op het verbeteren van het onderwijs voor zorgverleners, het vergroten van de toegang tot gespecialiseerde diensten en het implementeren van gestandaardiseerde zorgpaden.

In **Hoofdstuk 5** evalueren we de kwaliteit van leven (KvL) met behulp van de WHO-QOL-BREF-vragenlijst. We vonden dat de globale KvL bij mannen met KS aanzienlijk lager is vergeleken met een Britse referentiepopulatie. De globale KvL is het laagst onder mannen met KS die discriminatie hebben ervaren of lijden aan chronische psychische gezondheidsproblemen. Dit benadrukt de negatieve impact van discriminatie op het algehele welzijn van mannen met KS. Mannen met KS scoorden significant lager op zowel fysieke als psychologische KvL vergeleken met een gekoppelde Deense cohort. Een nieuwe bevinding van deze studie is dat mannen met KS minder deelnamen aan sociale activiteiten in vergelijking met de gezonde Europese referentiepopulatie en lagere scores rapporteerden in het sociale domein van de kwaliteit van leven. Dit kan te wijten zijn aan hogere niveaus van stress tijdens sociale interacties, schuchterheid en sociale angst onder mannen met KS.

In **Hoofdstuk 6** laten we zien dat mannen met KS lagere onderwijsniveaus en lagere beroepsstatus behalen en minder tevreden zijn met het inkomen in vergelijking met de referentiepopulatie van de Europese Sociale Enquête (ESS). Vertraging in de neurocognitieve ontwikkeling en een hoger percentage van zowel mentale als somatische morbiditeit dragen hier mogelijk aan bij. Onze structurele vergelijkingsmodellering (SEM) analyse liet een significante impact van het onderwijsniveau op de subjectieve algemene gezondheid zien. Het risico om geen beroep uit te oefenen was hoger voor mannen met KS in vergelijking met de ESS-referentiepopulatie. Redenen hiervoor waren een hoger risico op werkloosheid door permanent ziek of arbeidsongeschikt zijn, of met pensioen zijn. Onze SEM toonde aan dat een ernstiger fenotype van KS, een slechtere algemene gezondheidsstatus en de aanwezigheid van gezondheidsproblemen leidden tot een lagere beroepsstatus bij mannen met KS.

In **Hoofdstuk 7** bespreken we de onderzoeksvragen zoals geformuleerd in de introductie. De implicaties van de kennis die is verkregen uit de uitgevoerde studies voor de huidige klinische praktijk worden besproken. Toekomstig onderzoek moet zich richten op voorspellers van het aanwezig zijn van zaadcellen bij TESE bij volwassen mannen met KS. Daarnaast moet de zorg multidisciplinair worden aangepakt en vervolgens geëvalueerd, gebruik makend van geïntegreerde zorgmodellen vanwege de zeer variabele, verhoogde risico's van lichamelijke en geestelijke aandoeningen bij mannen met KS. Veel KS-patiënten melden lacunes in de toegang tot de gezondheidszorg en kennis van zorgverleners. KS-patiënten vertonen een significant lagere kwaliteit van leven in vergelijking met de algemene bevolking, waarbij fysieke, psychologische en sociale factoren bijdragen. KS-patiënten worden ook geconfronteerd met aanzienlijke sociaaleconomische uitdagingen, waaronder een lager opleidingsniveau en hogere werkloosheid, waarvoor gerichte ondersteuningsprogramma's nodig zijn. Er is een up-to-date evidence-based Europese richtlijn voor diagnose en behandeling van vruchtbaarheid bij mannen met KS. Een dergelijke interdisciplinaire, evidence-based en goed gestructureerde richtlijn ontbreekt echter nog voor de andere gebieden van de gezondheidszorg met betrekking tot KS, waarbij de puberale en cognitieve ontwikkeling, geavanceerde screeningonderzoeken en gezondheidsondersteuning aan bod komen om uiteindelijk de QOL en SES bij mannen met KS te verbeteren.



Appendices

Abbreviations
Bibliography
PhD Portfolio
Research data management
Curriculum vitae
Acknowledgements

Abbreviation

Abbreviation	Explanation
AMH	Anti-Müllerian Hormone
ART	Assisted Reproductive Technology
BMI	Body Mass Index
BMD	Bone Mineral Density
cTESE	Conventional Testicular Sperm Extraction
CSQ	Client Satisfaction Questionnaire
DSD	Disorders of Sex Development
E2	Estradiol
ELISA	Enzyme-Linked Immunosorbent Assay
ES cells	Embryonic Stem Cells
ESS	European Social Survey
FSH	Follicle-Stimulating Hormone
GP	General Practitioner
HR	Hazard Ratio
ICSI	Intracytoplasmic Sperm Injection
iPS cells	Induced Pluripotent Stem Cells
IVF	In Vitro Fertilization
KS	Klinefelter Syndrome
KvL	Kwaliteit van Leven (Quality of Life in Dutch)
LH	Luteinizing Hormone
mTESE	Microdissection Testicular Sperm Extraction
NGS	Next-Generation Sequencing
NIPT	Non-Invasive Prenatal Testing
NOA	Non-Obstructive Azoospermia
PAR1	Pseudoautosomal Region 1
PCR	Polymerase Chain Reaction
PRO	Patient Reported Outcome
QOL	Quality of Life
SCO	Sertoli Cell Only
SD	Standard Deviation
SEM	Structural Equation Modeling
SES	Socioeconomic Status
SHBG	Sex Hormone-Binding Globulin
SHOX	Short Stature Homeobox
SRY	Sex-determining Region Y
SSCs	Spermatogonial Stem Cells
TESE	Testicular Sperm Extraction
TRT	Testosterone Replacement Therapy
WHOQOL-BREF	World Health Organization Quality of Life - BREF

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PhD portfolio of Sebastian Franik

Department: Obstetrics and Gynaecology
PhD period: 01/04/2014 – 31/03/2025
PhD Supervisor(s): Prof. DDM Braat, Prof. H Claahsen-van der Grinten
PhD Co-supervisor(s): Dr. K Fleischer, Dr. L Ramos

Training activities	**Hours**
Courses	
• BROK Cursus (2015)	42.00
• RIHS - Introduction course for PhD candidates (2016)	15.00
• RIHS PhD introduction course (2016)	21.00
• Radboudumc - Scientific integrity (2016)	20.00
• Scientific Integrity for PhD candidates (2016)	28.00
Seminars	
• 12. Essener Symposium zur Gynäkologischen Onkologie und Senologie (2023)	0.20
• Update Gynäkologie Schloss Bensberg (2023)	0.20
• Drei-Säulen-Fortbildungsveranstaltung (2023)	0.10
Conferences	
• Pediatrics Endocrinology Symposium (oral, 2015)	14.00
• Fertility Research Meeting (oral, 2015)	14.00
• 28ste Symposium Experimenteel Onderzoek Heelkundige Specialismen (SEOHS) (2015)	14.00
• Department of Obstetrics and Gynaecology, Fertility Journal Clubs (2015)	84.00
• ECA Congress Rotterdam (poster, 2016)	42.00
• Department of Obstetrics and Gynaecology, Journal Club (2016)	84.00
• ESHRE 2017 Geneva (oral, 2017)	42.00
• IDSD Symposium Copenhagen (poster, 2017)	28.00
• DVR Conference Munich 2017 (poster, 2017)	28.00
• ESHRE Symposium 2018 Barcelona (oral, 2018)	42.00
• ESPE Conference Vienna 2019 (poster, 2019)	42.00
• ASRM Conference 2019 Philadelphia (oral, 2019)	42.00
• Journal Club Gynaecology, University of Muenster, 2017 - 2019 (2019)	252.00
Teaching activities	
Lecturing	
• Multiple Lectures and Student supervising at different gynaecology courses (2017)	14.00
• Multiple Lectures and Student supervising at different skill courses (2018)	42.00
• Multiple Lectures and student supervising at different courses (2019)	28.00
• Gyn Studienhospital (2022)	1.00
• Gyn-Praktikum 2 Sonography (2022)	0.10
• Gyn-Praktikum 2 clinical skills (2022)	0.10
Total	**939.70**

Research data management

Ethics and privacy

This thesis is based on the results of human studies, which were conducted in accordance with relevant national and international legislation and regulations, guidelines, codes of conduct and Radboudumc policy.

For Chapter 2, no ethical review board approval was obtained, as these studies did not involve human subjects or tissue.

The study conducted in Chapter 3 was approved by the local ethical committee for patient-related research, i.e. the Ethial committee Nijmegen, reference number: 2015-1614, date of approval: March 2015.

Chapters 4 - 6 are based on an international multicenter study, dsd-LIFE. Ethical approval was given by all institutional ethical boards of the participating centers and informed consent was provided by all participants. The study was registered in the German Clinical Trials Register: Registration identification number: DRKS00006072, date of registration April 17th, 2014. DRKS00006072 (German Clinical Trials Register). The privacy of the participants in these studies was warrented by the use of fully anonymous data.

Data collection and storage

There is no research Data for chapter 2.

Data for chapter 3 was obtained through laboratory experiments involving anonymous human materials. Raw data from laboratory systems are archived in their original form in a Data Acquisition Collection (DAC) in the Radboud Data Repository. Processed data and documentation (research protocol, experimental setup, codebook, software versions and a readme file) are archived in a Research Documentation Collection (RDC) in the Radboud Data Repository.

Data sharing according to the FAIR principles

Chapters 2 and 3 are published with restriced access. The datasets from chapter 3 are published in Data Sharing Collections (DSC's) in the Radboud Data Repository. The dataset from chapters 4 – 6 (dsd-LIFE study) will be published after en embargo. The dsd-LIFE datasets are not publicly available as long as primary analyses for other outcomes of dsd-LIFE are not completed. Afterwards scientific public use files are planned. The data will be made available by the principal investigator upon request to researchers after publication of the primary outcomes described in the grant by the consortium.

Chapter	DAC	RDC	DSC	DSC License
3	-	-	DOI:https://doi.org/10.34973/rver-sx54	CC-BY-NC
4	-	-	-	-
5	-	-	-	-
6	-	-	-	-

DAC = Data Acquisition Collection, RDC = Research Documentation Collection, DSC = Data Sharing Collection

Curriculum Vitae

◆Personal vita

Born in March, 22nd 1988 in Geldern (NRW)
Married to Lena Franik, having three kids: Charlotte *2018, Vincent *2019 and Tilda *2024
German

◆Occupation

07/2022 – Present	**Owner, Joint Practice "Frauenärzte am Niederrhein"** Specialist practice for gynecology and obstetrics, with a focus on specialized obstetrics and perinatology, prenatal diagnostics, as well as gynecological endocrinology and reproductive medicine.
12/2021 – 08/2023	**Medical Director, Coordination Unit for Covid-19 Vaccination, Kreis Kleve District** Appointed as the medical director of the coordination unit for Covid-19 vaccinations in the Kreis Kleve district.
10/2019 – 11/2021	**Resident Physician in Training in the Department of Gynecology and Obstetrics, Dr. Krebber's Practice, Kleve, North Rhine-Westphalia.** Specialist practice for gynecology and obstetrics, offering a comprehensive range of outpatient treatments in gynecology, gynecological oncology, obstetrics, urogynecology, gynecological endocrinology, and fertility treatments.
10/2017 – 09/2019	**Resident Physician in Training in the Department of Gynecology and Obstetrics, University Hospital Münster (UKM), North Rhine-Westphalia.** University hospital, level III endometriosis center, breast center, level I perinatal center with 1,500 births per year, fertility center. Full authorization for specialist training, including all sub-specialties.
04/2016 – 09/2017	**Resident Physician in Training in the Department of Gynecology and Obstetrics, St. Antonius Hospital, Kleve,**

North Rhine-Westphalia.
Regional hospital, breast center, obstetrics with 1,100 births per year, and neonatology on site. Full authorization for specialist training.

Education

10/2021	**Specialist in Gynecology and Obstetrics** Successfully passed the examination for Specialist in Gynecology and Obstetrics at the Medical Association of Düsseldorf.
02/2015 – current	**PHD project: Klinefelter and fertility** Department of Obstetrics and Gynaecology, Department of pediatric endocrinology, Radboudumc, Nijmegen *Promotor: Prof. Dr. D.D.M Braat*
03/2015 – (11/2015)	**“DedicatedSchakeljaar” gynaecology** Radboudumc, Nijmegen Catharina Ziekenhuis, Eindhoven
09/2012 – (12/2015)	**Study: Master human medicine** Radboud Universiteit, Nijmegen Finished with a degree of Master in medicine.
09/2011 – 08/2012	**Study: Pre-master medicine** Radboud Universiteit, Nijmegen A one-year combination of bachelor’s courses. Finished with a diploma “pre-master medicine”
09/2009 – 09/2011	**Radboud Honours Academy** Multidisciplinairy honours programme of the natural science faculty for most talented students.
09/2008 – 08/2011	**Study: B. Sc. (medical) biology** Radboud Universiteit, Nijmegen Major: Medical Biology. Minor: medicine Finished with a diploma Bachelor of Science (7.9), “bene merritum”

04/2008 – 07/2008	**Pre-study “medicine”** Rheinisches Bildungszentrum Köln Gmbh Preparation course for studying medicine, including anatomy, physiology, biology, physics, chemistry, maths
07/2007 – 03/2008	**Sozialer Dienst** St. Marien Krankenhaus, Kevelaer
08/1998 – 06/2007	**Kardinal-von-Galen-Gymnasium, Kevelaer** End-examination subjects: Biology, History, Maths, English

◆Medical conferences

10/2019	Speaker at the annual conference of the „American Society for reproductive medicine“ (ASRM) 2019 in Philadelphia.
09/2019	Poster presentation at the 58. Annual conference of the „European Society for Paediatric Endocrinology“ (ESPE) in Vienna.
07/2018	Speaker and participant at the annual conference of the „European Society of Human Reproduction and Embryology“ (ESHRE) in Barcelona
12/2017	Poster presentation at the 7. Congress of the „Dachverbands Reproduktionsbiologie und -medizin e.V.“ in München
07/2017	Speaker and participant at the annual conference of the „Europen Society of Human Reproduction and Embryology“ (ESHRE) in Genf
06/2017	Speaker at iDSD congress in Kopenhagen
09/2016	Poster presentation at the 9th European Congress of Andrology in Rotterdam.
09/2015	Poster Präsentation beim jährlichen Kongress der „Society for the Study of Inborn Errors of Metabolism“ (SSIEM)

05/2013	Speaker and participant at the annual **ESHRE** – European Society of Human Reproduction and Embryology conferece in London, United Kingdom
10/2012	Participant and staff member at the **FSA** – fertility society australia conferece in Auckland, New Zealand
09/2012	Participant and staff member at the 20th annual **Cochrane Colloquium** in Auckland, New Zealand
03/2010	Participating an international meeting about nanomedicine and presentation of a research protocol at the University of Oxford

◆Employment and periods abroad

Since 02/2014	Review author for the Cochrane Collaboration, Cochrane group for sexually transmitted infections
09/2012 – 12/2012	Scientific internship in Auckland, New Zealand with the Cochrane group for menstrual disorders and subfertility
Since 08/2012	Review author for the Cochrane Collaboration, Cochrane Group for Gynaecology and Fertility (CGFG)
05/2012	Cochrane workshop for writing systematic reviews in Oxford, United Kingdom
11/2010 – 01/2011	Internship at the “Department of Pathology” from the “University of Cambridge”, United Kingdom, finished with a “first” (85%)
07/2010 – 08/2010	Attending science summer school at Cambridge University (UK), Term I + II

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