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Onset and progression of puberty in Klinefelter syndrome

Mila Tanner[1,2] | Päivi J. Miettinen[1,2] | Matti Hero[1] | Jorma Toppari[3] | Taneli Raivio[1,2]

[1]New Children's Hospital, Pediatric Research Center, Helsinki University Hospital, Helsinki, Finland

[2]Research Programs Unit, Faculty of Medicine, Stem Cells and Metabolism Research Program, University of Helsinki, Helsinki, Finland

[3]Department of Pediatrics, Institute of Biomedicine, Research Centre for Integrative Physiology and Pharmacology and Centre for Population Health Research, Turku University Hospital, University of Turku, Turku, Finland

Correspondence
Taneli Raivio, Research Programs Unit, Faculty of Medicine, Stem Cells and Metabolism Research Program, University of Helsinki, PO Box 63, 00014, Helsinki, Finland.
Email: taneli.raivio@helsinki.fi

Abstract

Objective: Klinefelter syndrome (KS) (47,XXY and variants, KS) is the most common sex chromosome disorder in humans. However, little is known about the onset and progression of puberty in patients with KS. In this study, we describe the onset and progression of puberty in a large series of boys with KS in a single tertiary centre.

Design and Patients: Retrospective data (Tanner stages, testicular length, testosterone supplementation, levels of luteinizing hormone [LH] and testosterone) before possible testosterone treatment on 72 KS patients with 47,XXY karyotype were reviewed, and G (n = 59 patients) and P (n = 56 patients) stages were plotted on puberty nomograms.

Measurements and Results: One boy had a delayed onset of puberty, as he was at the G1 stage at the age of 13.8 years (−2.2 SDs). No observations of delay were made of boys at Stage G2. The progression of G stages was within normal limits in the majority of patients; only few boys were late at G3 (4.1%; 1 out of 24) and G4 (7.4%; 2 out of 27). Testosterone supplementation was started at the average age of 15.5 years to 35 boys (47%), 2 of whom were over 18 years old. LH level was on average 18.2 IU/L (SD: 6.3 IU/L) and testosterone 9.1 nmol/L (SD: 3.1 nmol/L) when testosterone supplementation was started.

Conclusions: Our results suggest that puberty starts within the normal age limits in boys with KS, and testosterone supplementation is not needed for the initial pubertal progression in the majority of patients.

KEYWORDS

androgens, Klinefelter syndrome, puberty, sex chromosome disorder, spermatogenesis, testis, testosterone

1 | INTRODUCTION

Klinefelter syndrome (KS) is the most common sex chromosome aneuploidy in men. It occurs approximately in one out of 660 males.[1] Males with KS have one or more extra X chromosomes in addition to normal male 46, XY karyotype. The additional X chromosome can be present in all or only in some of the cells. The classic form of KS (47,XXY) appears in 80%–90% of all the males with KS.[2]

KS is highly underdiagnosed due to large phenotypic variation. According to some studies, less than half of the males with KS become diagnosed during their lifetime.[1,3,4] In our unit, the average age at diagnosis of KS was 6.7 years.[4] Only a minority of boys with KS are

diagnosed prenatally, usually due to suspicion of other chromosome disorders than KS.[5] A good and efficient alternative to prenatal diagnosis is to involve KS in neonatal screening.[6] Early diagnosis of KS allows for appropriate treatment and helps to prevent possible complications in childhood and later in adolescence.[5,7]

In newborns with KS, the first signs can appear as microphallus and undescended testes.[8] Growth in height accelerates from the age of 3 years onward, and, on average, adult men with KS are taller than the general population.[9] Learning difficulties, delayed speech development, or problems in relationships with their peers and siblings during childhood are more frequent than in boys with normal karyotype.[10] Among boys with KS, gynaecomastia is common at pubertal age, but it is transient in most cases.[8,10] Testes are smaller in size already in childhood, and in puberty they fail to grow normally, which leads to small, firm testes in adulthood.[8,11] KS is the most common reason for male infertility, even though few spermatozoa can be found by testicular sperm extraction technique in more than 40% of males with KS.[12,13]

In childhood and early puberty, testosterone level stays rather normal in boys with KS while increasing degeneration of germ cells has been observed since the foetal period.[8,10–12] The number of germ cells decreases further during puberty when hyalinisation of seminiferous tubules begins and Sertoli cell degeneration and Leydig cell hyperplasia can be seen.[8,14,15] The serum levels of follicle-stimulating hormone (FSH) and especially luteinizing hormone (LH) start to increase in early puberty, which stimulates testosterone production.[16] Around mid-puberty, the response of Leydig cells to the increased level of LH fails, which leads to declining testosterone level and relative androgen deficiency.[8,10,17] Declining Leydig and Sertoli cell function ultimately leads to hypergonadotropic hypogonadism in which gonadotropin levels stay rather high while testosterone level is low. In addition, testes of KS patients have four times higher expression of aromatase activity than normal, leading to a high estradiol–testosterone ratio.[18]

Some data discrepancies can be found on the timing of the onset and progression of puberty in KS patients. Some researchers have reported on a delayed onset or progression of puberty in boys with KS,[5,14,15,17,19] while others claim that KS has no effect on the timing of puberty.[18,20–23] Therefore, more information about pubertal timing and the progression of boys with KS is needed. The aim of this retrospective study was to examine the onset and progression of puberty and testosterone supplementation in boys with KS in a single tertiary referral centre.

2 | PATIENTS AND METHODS

The data of the Children's Hospital, Helsinki University Hospital, a single tertiary centre of the Helsinki metropolitan area, were investigated from years 2004 to 2018. Altogether, 113 patients with KS born in 1967–2017 were found on the electronic patient registry by using the International Classification of Diseases 10 diagnoses listed in Table 1. Boys with mosaic KS or more than two X chromosomes and boys with no available data on pubertal development were excluded from the analysis. Altogether, 72 boys with classic KS (47,XXY), who were born between 1986 and 2006, were included in this study. Some data on a small subset of the boys have been published previously.[24] By the end of the data collection in 2018, 72% (52 out of 72) of boys with KS were over 18 years old. The study flow chart is represented in Figure 1.

The onset of central puberty is marked by the enlargement of testes at Tanner stage G2. The retrospective data included both Tanner genital (G, $n = 59$) and pubic hair (P, $n = 56$) stages and the lengths of the testes ($n = 39$) as well as serum LH, FSH and testosterone levels measured in the clinical laboratory. Testes were measured by a paediatrician with a ruler, a caliber or ultrasound.[25] The measurement method was neither registered nor observed in the analyses. All of these methods produce comparable results and are considered reliable.[25] The last recorded G1 and P1 stages and the first recorded G2–G5 and P2–P5 stages were considered in this study. Information about the delayed pubertal status of the parents, chronic diseases and other factors which may delay the onset of puberty in boys with KS, was also collected.

Figure 2 represents the number of boys with KS with available observations of pubertal progression. In total, 62 boys had observations of Tanner genital stages, pubic hair stages or testicular lengths; 35 boys had observations of them all. There were 10 boys with KS who did not have data on their Tanner G or P stages or testicular lengths. However, they had data on hormonal levels; five of which also had testosterone supplementation. Only data before testosterone supplementations were included in the study.

The details of Tanner genital and pubic hair stages were set to the Danish puberty nomogram[26] to visualize the pubertal progression of boys with KS. The Danish puberty nomogram was used because it has been shown to correspond well with the progression of pubertal stages of healthy Finnish boys in a small series from the 1980s[27] (data not shown). Testis lengths were set into a graph to visualize the early pubertal growth of testes. If the testes were different in size, the bigger one was chosen to the analysis. Testicular volumes in boys with KS were estimated graphically by using reference data on the relationship between testicular length and volume in healthy boys.[28] The volumes were rounded to the nearest testicular volume in the testicular nomogram.[26] Levels of testosterone, LH and FSH were set in a box and whisker plot according to the Tanner stages. Hormonal levels at the first achieved Tanner stage, either genital or pubic hair, were considered. If there were several measured hormonal levels at one Tanner stage, the mean value was considered. Medians of healthy boys' hormonal levels are demonstrated in the figure as control data.[29] Only data before any testosterone treatment were included.

TABLE 1 ICD-10 diagnoses used in the study

Q98.0	Klinefelter syndrome karyotype 47,XXY
Q98.1	Klinefelter syndrome, male with more than two X chromosomes
Q98.4	Klinefelter syndrome, unspecified
Q98.8	Other specified sex chromosome abnormalities, male phenotype

Abbreviation: ICD-10, International Classification of Diseases 10.

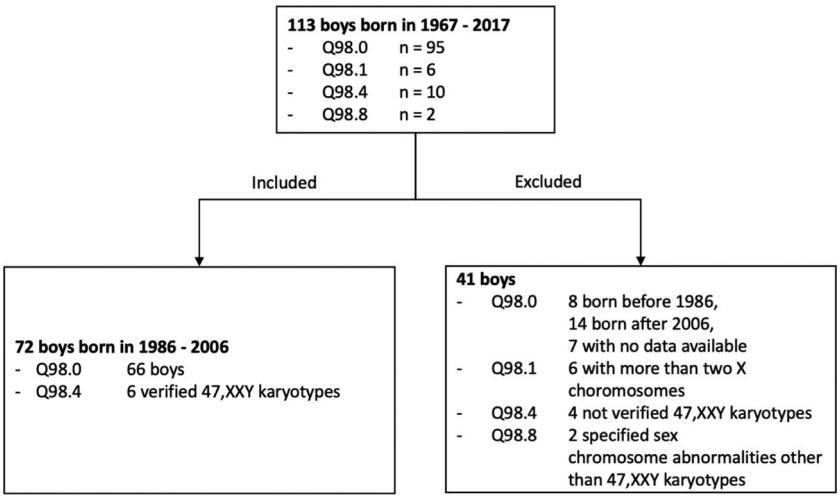
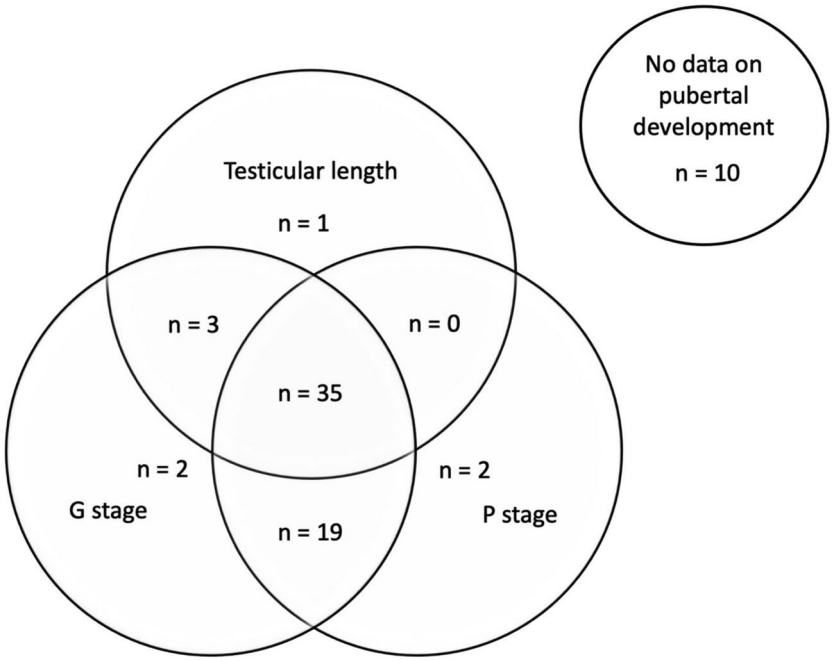


FIGURE 1 Flow chart of the patient data. We identified altogether 113 boys who were born between 1967 and 2017 in the original query conducted in the Helsinki University Hospital. Those born between 1986 and 2006 were included in the final analyses due to sufficient data on pubertal development. The ICD-10 codes are shown (Q98.0, Klinefelter syndrome karyotype 47,XXY; Q98.1, Klinefelter syndrome, male with more than two X chromosomes; Q98.4 Klinefelter syndrome, unspecified; Q98.8 other specified sex chromosome abnormalities, male phenotype). Only boys with Q98.0 and those with Q98.4 and verified karyotype were included. ICD-10, International Classification of Diseases 10

FIGURE 2 Venn diagram of the 72 boys with Klinefelter syndrome (KS). Circles represent patients with KS with available observations of their Tanner stages and testicular lengths. There were 62 boys with observations of Tanner genital stages, pubic hair stages or testicular lengths before testosterone supplementation. For the remaining 10 boys with KS, no data on pubertal progression were available. However, data on their hormonal levels and testosterone supplementation were available and included in the study



Information about testosterone supplementation of boys with KS was gathered to study the indications and timing of the supplementation. Serum levels of testosterone, LH and FSH as well as the age and Tanner genital stage at the onset of testosterone supplementation were collected.

Due to testosterone level changes during day and night, testosterone levels were measured before noon with the exception of one boy. Both testosterone and LH levels were measured from serum samples. The assays and their performance have been described previously.[30–34] In brief, testosterone was measured with radioimmunoassay (Lipidex-5000) until March 2005 with a sensitivity of 0.1 nM,[30] and the within and between assay coefficients of variation less than 5% and 7%, respectively.[30,31] Then a mass spectrometer method (API 2000 tandem mass spectrometer; AB Sciex) was used with a limit of detection 0.05 nmol/L, and interassay coefficient of variations (CVs) 4.2%–7.6% at mean concentrations of T of 3.3–45 nmol/L.[32] Gonadotropin levels were measured with immunofluorometric assay (AutoDELFIA; Wallac) until June 2011 with a

sensitivity of 0.019 IU/L for LH and 0.014 IU/L for FSH.[33] The interassay CVs, at concentrations of relevant to the current study, were less than 10%.[33] After that electrochemiluminescence immunoassay (Roche Diagnostics Gmbh) was utilized with the detection limit of LH and FSH of 0.1 IU/L and interassay CV less than 3% and 5%, respectively.[34]

2.1 | Statistics

Because this was a descriptive study, statistical tests were not employed.

3 | RESULTS

Almost all the boys with KS (n = 59) reached Tanner genital stages within the normal reference age range (Figure 3). Out of 17 boys with available observations for Stage G1, one boy remained late at the G1 stage and thus had a delayed onset of puberty. He had no history of familial delayed puberty. No observations of delay were made of boys at Tanner stage G2. At Tanner stage G3, one boy out of 24 (4%) had a delayed G3 stage (−2.7 SDs). For Tanner stage G4, 27 boys had observations available, and two of them (7%) were at G4 later than the reference boys (−2.2 SDs). The other one had a history of familial delayed puberty.

As demonstrated in Figure 4, one boy out of 25 stayed late at Tanner stage P1 (P1 stage at the age of 15 years; −2.3 SDs). He had no family history of delayed puberty. Out of 27 boys, two stayed late at the P3 stage (below −2.3 SDs). The other one had a history of familial delayed puberty. No other observations of delay were made of boys with KS in pubic hair progression.

Table 2 presents the number and average age of patients with available observations for at least two consecutive Tanner stages (Table 2a,b).

The testicular lengths of boys with KS (n = 39) are represented in Figure 5A. The graph indicates that the testes of boys with KS grew in early puberty, although the testicular lengths remained smaller than in healthy boys. Figure 5B represents the testicular volumes of each boy with KS plotted in a testicular nomogram. All boys 15 years or older had testicular volumes below −2 SDs.

Levels of testosterone, LH and FSH in relation to pubertal status are described in Figure 6. Median gonadotropin levels, especially that of FSH, exceed those in healthy boys in Tanner stage 3. Thereafter the picture of compensated hypogonadism emerges.

The average age of testosterone replacement therapy in 35 out of 72 boys, who had a record on the initiation of this treatment, was 15.5 years (range: 12.9–18.3 years). Testosterone was started on average, at Tanner stage G4. Only two of the 35 boys with KS were over 18 years old at the time of the first testosterone administration. At the onset of testosterone supplementation, the average LH level was 18.2 IU/L (SD: 6.3 IU/L) and testosterone level 9.1 nmol/L (SD: 3.1 nmol/L). The indications for testosterone supplementation were declining testosterone level (less than 10 nmol/L) and rising LH level (over 10 IU/L). All the boys with testosterone supplementation fulfilled at least one of these criteria. Of the 35 boys receiving testosterone supplementation, only one (3%) had a delayed clinical progression of puberty according to the puberty nomogram: He reached G4 stage at the age of 17.1 years and was given testosterone supplementation afterward.

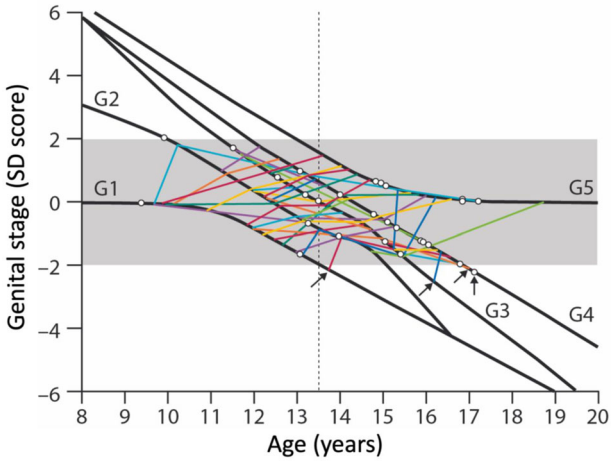


FIGURE 3 Tanner genital stages of boys with KS (n = 59) plotted in a Danish puberty nomogram.[26] G stages of each boy are combined with a line. The grey area represents the variation of healthy boys' genital stages (±2 standard deviations [SDs]). White dots represent boys with KS with only one available observation. Boys with delayed pubertal progression are marked with arrows. The age of delayed onset of puberty (−2 SD) is marked by a dotted line. Only data before any testosterone treatment are presented. KS, Klinefelter syndrome [Color figure can be viewed at wileyonlinelibrary.com]

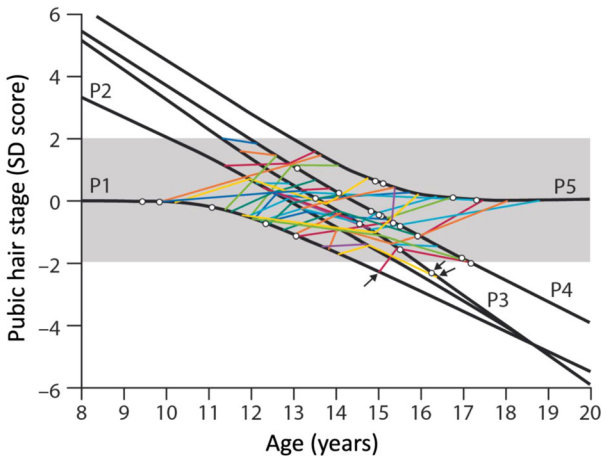


FIGURE 4 Tanner pubic hair stages of boys with KS (n = 56) plotted in a Danish puberty nomogram.[26] P stages of each boy are combined with a line. The grey area represents the variation of healthy boys' pubic hair stages (±2 standard deviations [SDs]). White dots represent boys with KS with only one available observation. Arrows show boys with delayed pubic hair progression. Only data before any testosterone treatment are presented. KS, Klinefelter syndrome [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Number of boys with KS and the age when (A) a new Tanner genital stage was recorded with the previous Tanner genital stage available and (B) a new Tanner pubic hair stage was recorded with the previous Tanner pubic hair stage available

	Patients (*n*)	Average age in years (range)
(A) *Tanner genital stage*[a]		
G2	12	12.8 (10.2–14.1)
G3	10	14.4 (12.9–15.5)
G4	8	15.7 (12.9–17.0)
G5	6	15.9 (14.2–17.2)
(B) *Tanner pubic hair stage*[b]		
P2	10	13.4 (11.9–14.7)
P3	11	14.11 (12.2–16.3)
P4	13	14.8 (12.2–17.0)
P5	9	15.8 (13.5–18.1)

Abbreviation: KS, Klinefelter syndrome.

[a]The average age in each Tanner genital stage is shown. See Figure 4 for normal age limits of G2–G5.

[b]The average age in each Tanner pubic hair stage is shown. See Figure 5 for normal age limits of P2–P5.

4 | DISCUSSION

In this study, we described the onset and progression of puberty in boys with KS by using a recently developed puberty nomogram to delineate normal timing and progression of pubertal events.[26] We found no apparent delay in the onset of puberty (age at G2) in subjects with KS, which is in agreement with the concept that the onset of puberty occurs normally in KS.[18,20–23,36,37] On the other hand, KS was associated with delayed puberty in the work by Sedlmeyer et al.[20] It is unclear if the boys with KS in Sedlmeyer's report had a history of familial delayed puberty or not.[20]

In the original work by Marshall and Tanner, the authors describe G2 to involve the enlargement of testes.[38] To help to delineate the onset of puberty in Finnish schools, the national guideline currently states that the testicular enlargement in G2 is defined as testicular length (measured with a ruler, a caliber, or ultrasound) of 25 mm or more.[28] While this cut-off works in practice, its applicability to boys with KS has not been tested. Our study suggests that the 25-mm rule to detect the onset of puberty also works for boys with KS, given their well-reported initial increase in testis size in puberty.[16,39] Tanner P stages and androgenic aspects of G3, G4 and G5 stages (i.e., penile development) are also useful in boys with KS, in accordance with their preserved Leydig cell function at this age.[14,15] Thus, the initial increase in testis size and the apparently normal androgenic capacity of the testes in early puberty render the diagnosis of KS during adolescence challenging and could explain why only 10% of boys with KS are diagnosed at the age of 14 years.[1] Indeed, the overtly small testis size may only be detected later in life in physical examinations carried out as a part of infertility investigation, for instance,[8,40] but this uncoupling of testicular development and virilization offers a diagnostic clue already in teenage.

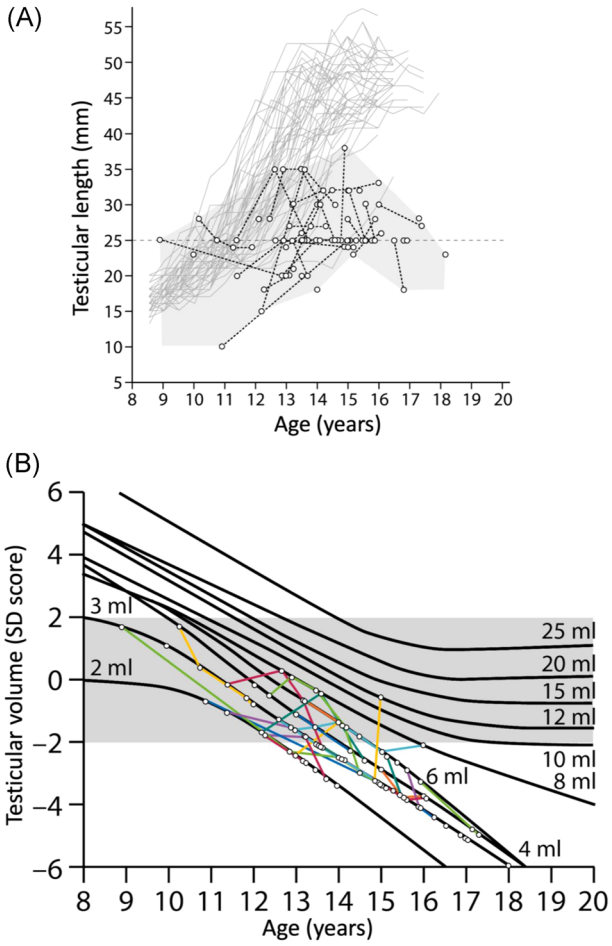


FIGURE 5 (A) Testicular lengths of each boy with KS are combined with a dotted line. Dots represent single observations of individual boys with KS. Grey area represents the range of testicular lengths and the horizontal dotted line represents the testicular length 25 mm which is accepted to denote the clinical onset of puberty (G2) in Finland.[35] Grey lines in the background represent testicular lengths of healthy boys according to the data in Sadov et al.[28] Only data before testosterone treatment are presented. (B) Testicular volumes in boys with KS were estimated by using testicular length as a surrogate (see Section 2 for details) and plotted in a testicular nomogram.[26] Testicular volumes of each boy are combined with a line. The grey area represents the variation of healthy boys' testicular volumes (±2 standard deviations [SDs]). KS, Klinefelter syndrome [Color figure can be viewed at wileyonlinelibrary.com]

Our study has clear limitations. First, we only report data before the onset of testosterone treatment. In Finland, testosterone treatment has been generally offered to boys with KS from mid puberty onwards if they have low levels of testosterone.[39] This treatment policy may have indeed biased the results. However, the age estimate for G2 in this study is devoid of this bias, since testosterone is never offered to boys with KS in G1, unless they exhibit constitutional delay of puberty (i.e., Tanner stage G1 at the age of 14 years or older without hypergonadotropic hypogonadism).[41] Furthermore, only two

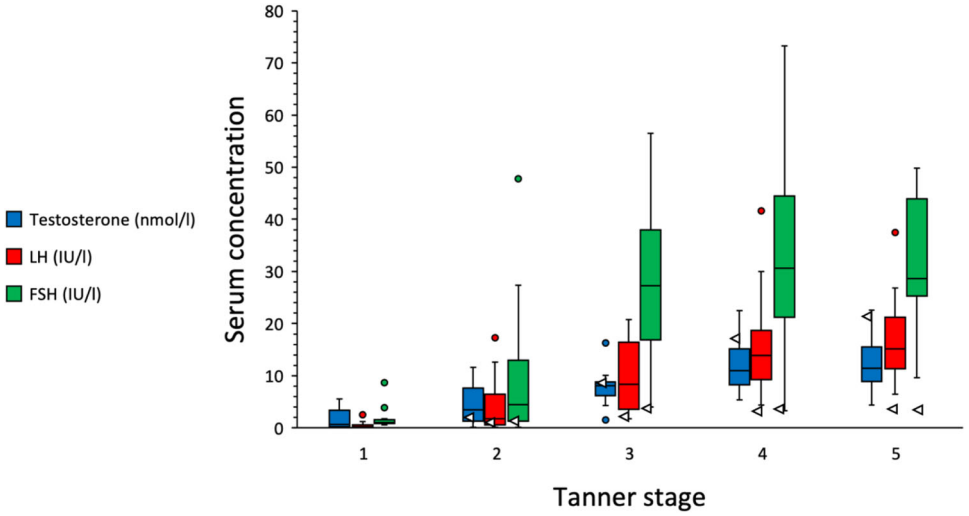


FIGURE 6 Reproductive hormones in relation to pubertal status presented in a box and whisker plot. Boxes span 50% of hormonal observations; the lower edge represents the first quartile, and the top edge represents the third quartile of the number of hormonal observations. The median value is marked by a horizontal line inside the box and the whiskers represent minimal and maximal values. Outliers are represented as points. Testosterone levels from 40 boys, and LH and FSH from 50 boys with KS are shown. The number of boys represented by each box and whisker ranges from 9 to 30 (average 17). The open arrowheads represent healthy boys' medians of respective hormone levels in Tanner stages 2–5.[29] Only data before any testosterone treatment are included. FSH, follicle-stimulating hormone; KS, Klinefelter syndrome; LH, luteinizing hormone [Color figure can be viewed at wileyonlinelibrary.com]

of the 35 patients with KS (5.7%), who eventually received testosterone supplementation, had a slow clinical progression of puberty according to the Tanner genital stage nomogram at the onset of testosterone supplementation. Finally, different testicular measurement methods were employed, which may be an additional source of variation.[28] On the other hand, our results reflect everyday clinical practice in a single tertiary centre.

Our results suggest that testosterone supplementation is not needed for normal pubertal progression in the majority of patients with KS, yet there is evidence of its benefits in adulthood.[42,43] In hypogonadal males with KS, testosterone supplementation improves bone mineral density at the lumbar spine and body composition by increasing lean mass and reducing fat mass.[44] In prepubertal boys with KS, the levels of testosterone may be subnormal when assessed with more sensitive methods.[45,46] However, there is no clear consensus on the benefits of testosterone supplementation before puberty. Few studies report that androgen treatment may have a positive effect on neurodevelopment in infants with KS.[47,48] On the other hand, it also increases the risk of early gonadarche.[49] European Academy of Andrology (EAA)[50] has created guidelines on KS based on the Grading of Recommendations, Assessment, Development and Evaluation system. Testosterone treatment is not recommended by the EAA for infants or prepubertal boys with KS because of the low number and lack of the reliability of studies devoted to the subject.[50] However, in infants with micropenis, a low-dosed systemic testosterone treatment may be provided.[50]

In conclusion, our results suggest that puberty starts within the normal age limits in boys with KS. Physicians treating these patients should be aware of the initial testicular enlargement in puberty, which appears to efficiently mask the subsequent deterioration of seminiferous epithelium function and the development of hypergonadotropic hypogonadism. Neonatal screening[51] could help to diagnose boys with KS in a timely fashion and allow personalized approach during childhood and adolescence.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

M.T. collected clinical data, analyzed it and drafted the manuscript; P.J.M., M.H., and J.T. analyzed data and drafted the manuscript; T.R. analyzed the data, drafted the manuscript and coordinated the study. All authors critically revised the manuscript.

ETHICS STATEMENT

The study was approved by the Ethics Committee of Helsinki University Hospital. Because this study was based solely on health record data, informed consents were not required. Helsinki University Hospital approved the study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Mila Tanner http://orcid.org/0000-0001-6487-7854

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