Endocrine and metabolic evaluation of classic Klinefelter syndrome and high-grade aneuploidies of sexual chromosomes with male phenotype: are they different clinical conditions?

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Abstract

Objective: Klinefelter syndrome (KS) is the most common sex chromosome aneuploidy in males. As well as classic KS, less frequent higher-grade aneuploidies (HGAs) are also possible. While KS and HGAs both involve testicular dysgenesis with hypergonadotropic hypogonadism, they differ in many clinical features. The aim of this study was to investigate the endocrinal and metabolic differences between KS and HGAs.
Design: Cross-sectional, case-control study.
Methods: 88 patients with KS, 24 with an HGA and 60 healthy controls. Given the known age-related differences all subjects were divided by age into subgroups 1, 2 and 3. Pituitary, thyroid, gonadal and adrenal functions were investigated in all subjects. Metabolic aspects were only evaluated in subjects in subgroups 2 and 3.
Results: FT4 and FT3 levels were significantly higher in HGA than in KS patients in subgroups 1 and 2; in subgroup 3, FT4 was significantly higher in controls than in patients. Thyroglobulin was significantly higher in HGA patients in subgroup 1 than in KS patients and controls. Hypergonadotropic hypogonadism was confirmed in both KS and HGA patients, but was more precocious in the latter, as demonstrated by the earlier increase in gonadotropins and the decrease in testosterone, DHEA-S and inhibin B. Prolactin was significantly higher in HGA patients, starting from subgroup 2. Total and LDL cholesterol were significantly higher in HGA patients than in KS patients and controls, while HDL cholesterol was higher in controls than in patients.
Conclusions: KS and HGAs should be considered as two distinct conditions.

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Introduction

Klinefelter syndrome (KS), described for the first time by Harry F. Klinefelter in 1942 (1), is the most common form of male sex chromosome aneuploidy, with an estimated frequency of 1:500–1:1000 in the male population (2). It is the consequence of a nondisjunction mechanism, which occurs during paternal or maternal meiosis, leading to the classic 47,XXY karyotype (3). In adults, it typically presents with tall stature, small testes, sparse body hair, gynaecomastia, gynoid appearance, hypergonadotropic hypogonadism, hyalinization and fibrosis of the

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seminiferous tubules and, consequently, azoospermia (4, 5, 6, 7, 8, 9, 10). As well as the classic 47,XXY form of KS, higher-grade aneuploidies (HGAs) are possible: 48,XXYY, the most common, with an incidence of 1:18 000–1:40 000 (11); 48,XXXY, seen in about 1:50 000 males (12); 49,XXXXY, whose incidence is near 1:85 000–1:100 000 (12) and 49,XXXYY, whose real incidence is currently unknown, with only six cases reported in the literature.

The first description of an HGA dates back to an article by Marco Fraccaro published in The Lancet in 1960 (13), which described a 7-year-old child with 49 chromosomes. These very rare conditions all involve the testicular dysgenesis associated with hypergonadotropic hypogonadism typical of KS, and for this reason, are usually considered as variants of KS (14), even though they have their own distinct aspects: dysmorphic facial features, skeletal deformities, hypotonic musculature, tremors, genital anomalies and neurologic and cognitive impairment (15, 16, 17, 18, 19). The aim of this study was to investigate, for the first time in the scientific literature, the endocrinal and metabolic differences between classic 47,XXY KS and the HGAs, in order to obtain sufficient objective data to consider them as different conditions.

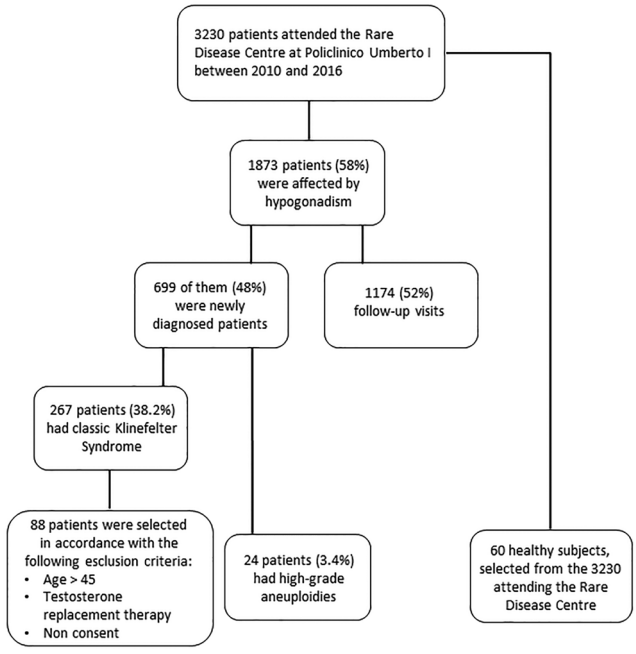

Figure 1
Flow chart of the recruitment process for patients and control subjects attending the Rare Disease Centre at Umberto I Policlinico between 2010 and 2016.

Subjects and methods

We selected 1873 hypogonadal patients and 60 healthy subjects from a cohort of 3230 subjects attending the Rare Disease Centre at Umberto I Policlinico between March 2010 and April 2016. Of the hypogonadal patients, 699 (58%) were newly diagnosed: of these, 267 (38.2%) were diagnosed with KS, while 24 (3.4%) were affected by HGAs. The exclusion criteria of testosterone replacement therapy, age >45 years and non-consent, led to the elimination of a further 179 KS patients, while no HGA patients had to be excluded (Fig. 1).

Consequently, eighty-eight 47,XXY patients (KS group: age 2.5–44 years; mean age ± S.D.: 23 ± 10), twenty-four patients with higher-grade aneuploidies (HGA group: age 1.9–34 years; mean age ± S.D.: 17 ± 9.4) and sixty age-matched healthy subjects (control group: age 3.5–40; mean age ± S.D.: 24 ± 9.7) underwent laboratory hormone tests. Patients in the HGA group had the following karyotypes: four with 48,XXXY; twelve with 48,XXYY and eight with 49,XXXXY.

Pituitary, thyroid, gonadal and adrenal function was studied by measurement of the following hormones: thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), thyroglobulin (Tg), follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (T), 17 beta-estradiol (E2), inhibin B (INHB), sex hormone-binding globulin (SHBG), prolactin (PRL), delta 4-androstenedione (Δ4) and dehydroepiandrosterone sulfate (DHEA-S).

As already noted, at the time of the study, none of the subjects were taking testosterone replacement therapy or other endocrine-active drugs.

Given the age-related differences in the endocrine system, the subjects were divided into three further subgroups by age: subgroup 1 (age ≤12 years: 12 KS patients aged 2.5–10.9 years; mean age ± S.D.: 6.6 ± 2.6; 8 HGA patients aged 1.9–11.8 years; mean age ± S.D.: 6.9 ± 2.8; 15 controls aged 3.5–11.6 years; mean age ± S.D.: 7.3 ± 3.2); subgroup 2 (age >12 and ≤20 years: 26 KS patients aged 12.9–19.8 years; mean age ± S.D.: 17.1 ± 1.8; 8 HGA patients aged 12.8–19.7 years; mean age ± S.D.: 16.7 ± 2.3; 20 controls aged 12.1–19.9 years; mean age ± S.D.: 18 ± 2) and subgroup 3 (age >20 years: 50 KS patients aged 20.7–44 years; mean age ± S.D.: 30.2 ± 6.7; 8 HGA patients aged 20.8–34 years; mean age ± S.D.: 26.6 ± 4.4; 25 controls aged 21.1–40 years; mean age ± S.D.: 29.9 ± 5.9). There were no statistically significant differences in age between the subgroups. Investigation of Tanner pubertal stage revealed the following: KS patients: 7 G1 and 5 G2 in subgroup

1; 8 G3, 12 G4 and 6 G5 in subgroup 2; all G5 in subgroup 3. HGA patients: 5 G1 and 3 G2 in subgroup 1; 1 G2, 3 G3 and 4 G4 in subgroup 2 and, as with the KS patients, all G5 in subgroup 3.

Subjects in subgroups 2 and 3 also underwent an evaluation of lipid and glucose metabolism, with the analysis of total, LDL and HDL cholesterol, triglycerides, basal blood glucose, 2-h glucose (after OGTT), basal insulin, 2-h insulin (after OGTT), HOMA index and glycated haemoglobin.

Hormone assay

Baseline blood samples were obtained from all subjects by antecubital venous puncture in the early morning (07:30–09:00 h) after an overnight fast. Samples were centrifuged after 30′ and the serum was immediately frozen at −20°C.

TSH, FT3, FT4, FSH, LH, SHBG, PRL, DHEA-S, E2 and T were measured in duplicate with chemiluminescent microparticle immunoassay (CMIA, Architect System; Abbott Laboratories) with limits of detection (LOD) of ≤0.0025 μIU/mL, ≤1.0 pg/mL, ≤0.4 ng/dL, 0.05 IU/mL, 0.07 IU/mL, ≤0.1 nmol/L, 0.25 ng/mL, ≤3.0 μg/dL, ≤10 pg/mL and 0.28 nmol/L respectively. Intra- and inter-assay coefficients of variation for our laboratory were: 3.6 and 5.4% at 2.71 μIU/mL (TSH); 2.8 and 5.2% at 2.76 pg/mL (FT3); 3.1 and 5.1% at 1.07 ng/dL (FT4); 3.6% and 5.4% at 3.2 IU/mL (FSH); 3.8 and 5.5% at 4.1 IU/mL (LH); 5.65% and 9.54% at 8.8 nmol/L (SHBG); 3.32% and 6.92% at 4.19 ng/mL (PRL); 2.68% and 7.41% at 10.7 μg/dL (DHEA-S); 5% and 7% at 190 and 600 pg/mL (E2) 2.1% and 3.6% at 10.08 nmol/L (T). Serum Tg was measured by chemiluminescent assay (ACCESS; Beckman Coulter Laboratories, Brea, CA,USA) with a LOD of 0.1 ng/mL and intra- and inter-assay coefficient of variation <10% at >1 ng/mL (20, 21). Serum concentrations of INHB were measured by enzyme-linked immunosorbent assay (ELISA) (GEN II, Beckman Coulter laboratories) with LOD of 7.0 pg/mL, while intra- and inter-assay coefficients of variation were 3.3% and 7.2% at 122 pg/mL. Finally Δ_4 was measured with radioimmunoassay (RIA) (Cisbio Bioassays, Codolet, France) with LOD 5 ng/dL; intra- and inter- assay coefficients of variation were 7.4% and 8.1% at 168 ng/dL.

The reference ranges for pre-pubertal subjects were 0.87–5.19 μIU/mL for TSH; 3.09–5.59 pg/mL for FT3; 1.01–1.60 ng/dL for FT4; <0.05–2.00 IU/mL for FSH; <0.07–1.80 IU/mL for LH; <0.28–2.2 nmol/L for T; 16.50–242.80 μg/dL for DHEA-S. Normal ranges for pubertal adolescents were 0.76–4.51 μIU/mL for TSH; 2.77–5.51 pg/mL for FT3; 0.85–1.48 ng/dL for FT4. Finally, the reference ranges for adulthood were 0.49–3.87 μIU/mL for TSH; 2.32–4.31 pg/mL for FT3; 0.81–1.34 ng/dL for FT4; 1.15–45.00 ng/mL for Tg; 1.38–9.58 IU/mL for FSH; 1.79–8.17 IU/mL for LH; 10.40–38.20 nmol/L for T; 24–108 pg/mL for E2; 80–380 pg/mL for INHB; 11.1–78.2 nmol/L for SHBG; 2.63–13.14 ng/mL for PRL; 130–286 ng/dL for Δ_4; 133.1–592.00 μg/dL for DHEA-S.

Total cholesterol, HDL cholesterol and triglycerides were measured by an enzymatic colorimetric assay (respectively Roeschlau, 1974 – Sugiuchi, 1995 – Siedel, 1993), while LDL cholesterol was calculated according to Friedwald's equation. Glucose was measured by the hexochinase-G6PD method (UV-Kunst, 1984), while insulin was determined by an electrochemiluminescent assay (Clark, 1999). Glycated haemoglobin was assessed by high-performance liquid chromatography.

This study was approved by the Institutional Ethics Committee.

Statistical analysis

Data are expressed as mean ± S.D. or median and 95% confidence interval. To enable the statistical analysis, when INHB, FSH, LH and T were below the LOD, the value of respectively 4 pg/mL, 0.03 IU/mL, 0.04 IU/mL and 0.20 nmol/L were used arbitrarily. Comparisons between two groups were carried out by Student *T* test for parametric analysis and the Mann–Whitney *U* test for non-parametric data. Comparisons among three groups were performed through ANOVA variance analysis, while the differences in medians, for non-parametric analysis, were calculated using the Kruskal–Wallis test. Statistical significance is given as a 95% confidence interval ($P<0.05$).

Results

All results are reported as mean ± S.D. unless otherwise indicated.

Pituitary-testis axis and DHEA-S

Subgroup 1

Blood serum concentrations of FSH and DHEA-S were significantly higher in HGA than in KS patients (FSH: 1.5 ± 0.83 vs 0.37 ± 0.14 IU/mL; $P<0.005$; DHEA-S: 44 ± 11

vs 25±20 µg/dL; $P<0.05$) and FSH was higher in HGA patients than controls (1.5±0.83 vs 0.71±0.44 IU/mL; $P<0.005$) (Figs 2 and 3).

Subgroup 2

There were significant differences in LH and FSH between KS patients and controls (LH: 11±5.6 vs 3.2±1.0 IU/mL; $P<0.0001$; FSH: 24±12 vs 3.5±1.9 IU/mL; $P<0.0001$), between HGA patients and controls (LH: 15±4.6 vs 3.2±1.0 IU/mL; $P<0.0001$; FSH: 35±10 vs 3.5±1.9 IU/mL; $P<0.0001$) and between KS and HGA patients (LH: 11±5.6 vs 15±4.6 IU/mL; $P<0.05$; FSH: 24±12 vs 35±10 IU/mL; $P<0.05$) (Fig. 2). It is clear that gonadotropin levels were higher in HGA than in KS patients, and both had higher concentrations than seen in age-matched controls.

The following results and significance levels were observed for T; HGA vs controls: 8.5±4.0 vs 20±6.1 nmol/L; $P<0.0001$; KS vs controls: 15±4.5 vs 20±6.1 nmol/L; $P<0.005$; HGA vs KS: 8.5±4.0 vs 15±4.5 nmol/L; $P<0.05$ (Fig. 4). For INHB the results were as follows: HGA vs controls: 5.3±1.5 vs 162±67 pg/mL; $P<0.0001$; KS vs controls: 18±15 vs 162±67 pg/mL; $P<0.0001$; HGA vs KS: 5.3±1.5 vs 18±15 pg/mL; $P<0.05$ (Fig. 4).

There was a significant difference in levels of the adrenal hormone DHEA-S between KS and HGA patients (203±112 vs 124±67 µg/dL; $P<0.05$), between KS patients and controls (203±112 vs 387±120 µg/dL; $P<0.05$) and between HGA patients and controls (124±67 vs 387±120 µg/dL; $P<0.01$) (Fig. 3). For SHBG and PRL (Figs 3 and 4), significant differences were seen between HGA patients and controls (SHBG: 48±21 vs 31±13 nmol/L; $P<0.05$; PRL: 22±12 vs 7.3±2.0 ng/mL; $P<0.005$), while there was a significant difference

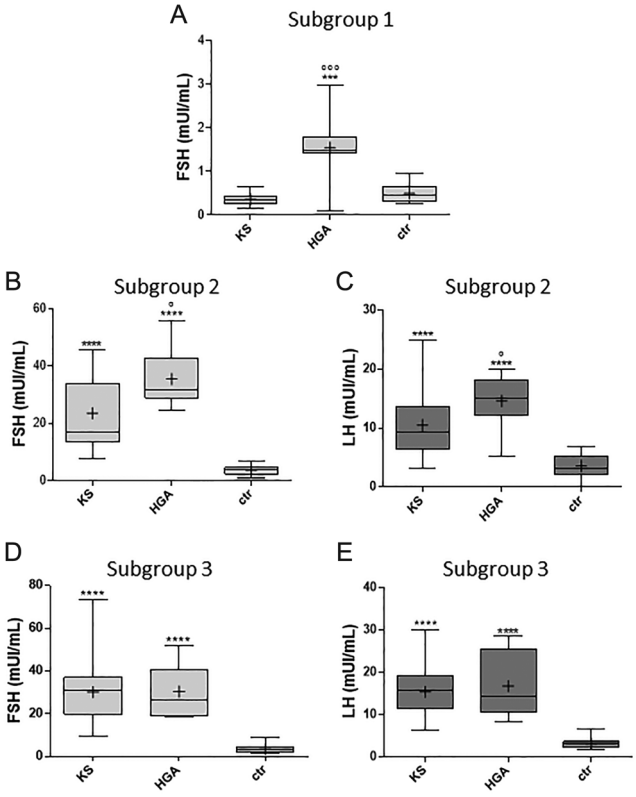

Figure 2
Box plots of gonadotropins in patients and control subgroups. Median, minimum and maximum values (whiskers) are reported for subgroups 1, 2 and 3. Mean (+) and ANOVA *P* values are also reported: (A, B and D) FSH plasma levels. °°°$P<0.005$ vs KS and ***$P<0.005$ vs controls; °$P<0.05$ vs KS; ****$P<0.0001$ vs controls. (C and E) LH plasma levels. ****$P<0.0001$ vs controls; °$P<0.05$ vs KS.

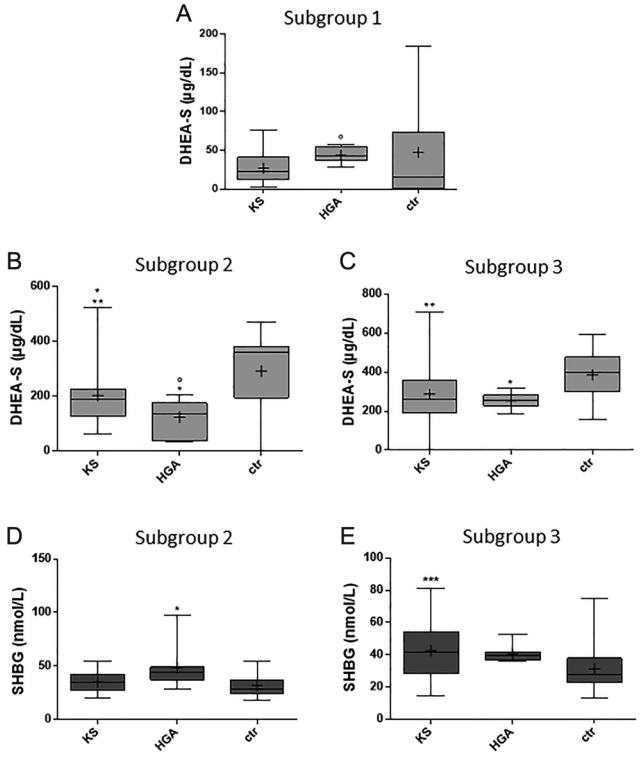

Figure 3
Box plots of DHEA-S and SHBG in patients and control subgroups. Median, minimum and maximum values (whiskers) are reported for subgroups 1, 2 and 3. Mean (+) and ANOVA *P* values are also reported: (A, B and C) DHEA-S plasma levels. *$P<0.05$ vs controls; **$P<0.01$ vs controls; °$P<0.05$ vs KS. (D and E) SHBG plasma levels. ***$P<0.001$ vs controls; *$P<0.05$ vs controls.

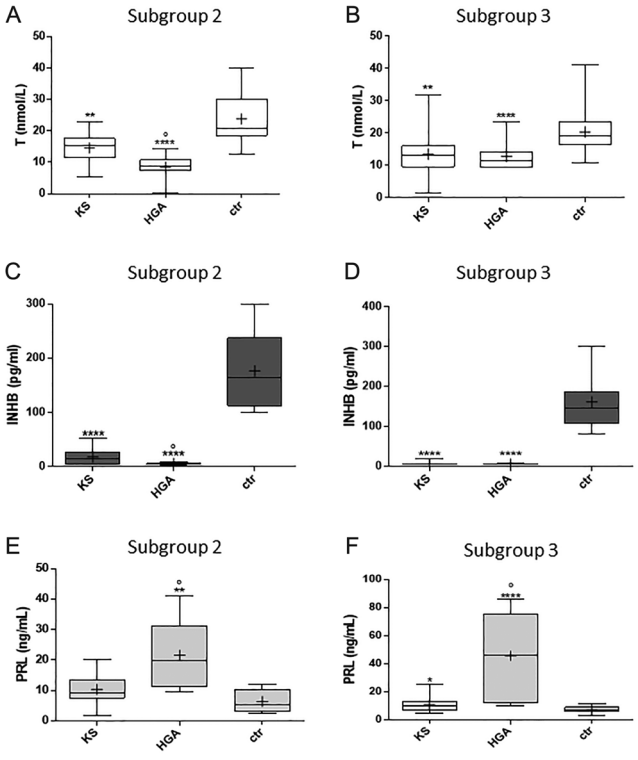

Figure 4

Box plots of testosterone, inhibin B and prolactin in patients and control subgroups. Median, minimum and maximum values (whiskers) are reported for subgroups 1, 2 and 3. Mean (+) and ANOVA *P* values are also reported: (A and B) Testosterone plasma levels. ****$P<0.0001$ vs controls; **$P<0.005$ vs controls; °$P<0.05$ vs KS. (C and D) Inhibin B plasma levels. ****$P<0.0001$ vs controls; °$P<0.05$ vs KS. (E and F) Prolactin plasma levels. ****$P<0.0001$ vs controls; **$P<0.005$ vs controls; *$P<0.05$ vs controls; °$P<0.05$ vs KS.

between KS and HGA patients in PRL alone (10±5.0 vs 22±12 ng/mL; $P<0.05$).

Subgroup 3

There was a significant difference in FSH and LH levels between KS patients and controls (FSH: 30±14 vs 3.5±1.9 IU/mL; $P<0.0001$; LH: 15±5.6 vs 3.2±1.0 IU/mL; $P<0.0001$) and between HGA patients and controls (FSH: 31±13 vs 3.5±1.9 IU/mL; $P<0.0001$; LH: 17±7.7 vs 3.2±1.0 IU/mL; $P<0.0001$) (Fig. 2). DHEA-S, T and INHB (Figs 3 and 4) were higher in controls than in KS patients (DHEA-S: 387±120 vs 288±150 µg/dL; $P<0.05$; T: 20±6.1 vs 14±6.5 nmol/L; $P<0.005$; INHB: 162±67 vs 5.5±3.1 pg/mL; $P<0.0001$) and HGA patients (DHEA-S: 387±120 vs 254±41 µg/dL; $P<0.05$; T: 20±6.1 vs 13±4.7 nmol/L; $P<0.0001$; INHB: 162±67 vs 5.6±1.1 pg/mL; $P<0.0001$). SHBG levels were higher in KS patients than controls (43±17 vs 31±13 nmol/L; $P<0.001$) (Fig. 3). Finally, there was a significant difference in PRL between KS patients and controls (11±4.9 vs 7.3±2.0 ng/mL; $P<0.05$), between KS and HGA patients (11±4.9 vs 46±32 ng/mL; $P<0.05$) and between HGA patients and controls (46±32 vs 7.3±2.0 ng/mL; $P<0.0001$).

There were no significant differences in E2 and Δ4 between controls and patients in any of the subgroups (Table 1).

Pituitary-thyroid axis

Before describing the results, it must be stressed that all thyroid hormone levels in all subjects (KS, HGA and controls) fell within the normal range.

Subgroup 1

There was a significant difference between KS and HGA patients in FT4 (1.0±0.08 vs 1.2±0.14 ng/dL; $P<0.05$) and Tg (12±6.5 vs 26±4.9 ng/mL; $P<0.05$). There was also a significant difference between HGA patients and controls in Tg (26±4.9 vs 14±6.2 ng/mL; $P<0.005$) (Table 1).

Subgroup 2

FT3 levels were significantly higher in HGA than KS patients (3.7±0.32 vs 3.3±0.26 pg/mL; $P<0.05$) (Table 1).

Subgroup 3

There were significant differences in FT4 between KS patients and controls (1.0±0.15 vs 1.13±0.10 ng/dL; $P<0.005$) and between HGA patients and controls (0.95±0.09 vs 1.13±0.10 ng/dL; $P<0.0005$) (Table 1).

Lipid profile

As already noted, the lipid profile investigation involved the analysis of total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides in subgroups 2 and 3 (Table 2).

Subgroup 2

Total and LDL cholesterol were significantly higher in HGA than in KS patients (total: 160±6.7 vs 141±18 mg/dL;

Table 1 Thyroid hormone profile, 17 beta-estradiol and delta 4-androstenedione (Δ4) mean values in patients and control groups. Data are reported as mean ± S.D.

	KS	HGA	Controls
Subgroup 1 (age ≤12)			
n	12	8	15
TSH (μIU/mL)	2.1 ± 1.1	2.5 ± 1.4	2.4 ± 1.4
FT3 (pg/mL)	3.7 ± 0.36	3.8 ± 0.41	4.0 ± 0.50
FT4 (ng/dL)	1.0 ± 0.08	1.2 ± 0.14[a]	1.1 ± 0.12
Tg (ng/mL)	12 ± 6.5	26 ± 4.9[a,b]	14 ± 6.2
E2 (pg/mL)	15.6 ± 8.5	11 ± 2.7	10.5 ± 1.2
Δ4 (ng/dL)	25.8 ± 21.9	34 ± 6.1	38.6 ± 28.5
Subgroup 2 (12 < age ≤ 20)			
n	26	8	20
TSH (μIU/mL)	1.6 ± 0.97	1.5 ± 0.92	2.1 ± 0.34
FT3 (pg/mL)	3.3 ± 0.26	3.7 ± 0.32[a]	3.6 ± 0.53
FT4 (ng/dL)	0.98 ± 0.14	1.1 ± 0.06	1.1 ± 0.10
Tg (ng/mL)	13 ± 9.8	16 ± 10	8.2 ± 3.7
E2 (pg/mL)	25 ± 10.2	21 ± 10.2	25.5 ± 8.8
Δ4 (ng/dL)	204.9 ± 84.2	176 ± 88.9	195 ± 8.8
Subgroup 3 (age >20)			
n	50	8	25
TSH (μIU/mL)	1.5 ± 0.79	1.6 ± 0.30	1.52 ± 0.69
FT3 (pg/mL)	3.1 ± 0.35	3.0 ± 0.28	3.11 ± 0.29
FT4 (ng/dL)	1.0 ± 0.15[b]	0.95 ± 0.09[c]	1.13 ± 0.10
Tg (ng/mL)	19 ± 22	24 ± 26	8.77 ± 6.13
E2 (pg/mL)	34 ± 19.4	26 ± 7.8	30.2 ± 14.7
Δ4 (ng/dL)	223.1 ± 109.9	177 ± 53	182.4 ± 46.5

[a]$P<0.05$ vs KS, [b]$P<0.005$ and [c]$P<0.0005$ vs controls.
Controls, healthy subjects; HGA, high-grade aneuplodies' patients; KS, Klinefelter syndrome's patients.

$P<0.05$; LDL: mean ± S.D.: 99 ± 6.9 vs 75 ± 17 mg/dL; $P<0.005$) and controls (total: 160 ± 6.7 vs 128 ± 26 mg/dL; $P<0.05$; LDL: 99 ± 6.9 vs 47 ± 11 mg/dL; $P<0.0005$). LDL cholesterol was significantly higher in KS patients than in controls (75 ± 17 vs 47 ± 11 mg/dL; $P<0.0005$). In contrast, HDL cholesterol concentrations were higher in controls than in KS and HGA patients (controls vs KS: 74 ± 22 vs 54 ± 13 mg/dL; $P<0.01$; controls vs HGA: 74 ± 22 vs 43 ± 5.8 mg/dL; $P<0.005$). There were no significant differences in triglycerides in this subgroup (Table 2).

Subgroup 3

The results in this subgroup are generally similar to those for subgroup 2. Total cholesterol was significantly higher in KS and HGA patients than controls (KS vs controls: 181 ± 30 vs 126 ± 18 mg/dL; $P<0.0001$; HGA vs controls: 211 ± 49 vs 126 ± 18 mg/dL; $P<0.0001$). The results for LDL cholesterol reflect those for subgroup 2 (KS vs controls: 107 ± 28 vs 45 ± 13 mg/dL; $P<0.0001$; HGA vs controls: 147 ± 46 vs 45 ± 13 mg/dL; $P<0.0001$). LDL concentrations were also higher in HGA than in KS patients (147 ± 46 vs 107 ± 28 mg/dL; $P<0.05$). Finally, HDL cholesterol was higher in controls than in KS and HGA patients (controls vs KS: 64 ± 17 vs 52 ± 17 mg/dL; $P<0.05$; controls vs HGA: 64 ± 17 vs 46 ± 6.5 mg/dL; $P<0.05$). Here too, there were no significant differences in triglycerides (Table 2).

Glucose profile

Evaluation of glucose profile included OGTT, insulin, HOMA index and glycated haemoglobin. No statistically significant differences were seen between KS patients, HGA patients and controls. Although higher values for these parameters were seen in HGA than KS patients in subgroup 2, this trend was almost completely reversed in subgroup 3 (Table 2).

Discussion

To our knowledge, the present study is the first to evaluate endocrinal and metabolic features in HGA patients and the differences between such patients and patients with classic 47,XXY KS. It is also the first to perform a triple comparison among classic KS, HGA and control subjects.

Table 2 Lipid profile, OGTT, HOMA index and glycated haemoglobin values in patients and control groups. Data are reported as mean ± S.D. or median and lower and upper CI 95%.

	KS	HGA	Controls
Subgroup 2 (12 < age ≤ 20)			
n	26	8	20
Total cholesterol (mg/dL)	141 ± 18	160 ± 6.7[a,b]	128 ± 26
LDL (mg/dL)	75 ± 17[f]	99 ± 6.9[e,f]	47 ± 11
HDL (mg/dL)	54 ± 13[c]	43 ± 5.8[d]	74 ± 22
Triglycerides (mg/dL)	66; 58–87	90; 50–114	60; 49–83
Basal glucose (mg/dL)	82; 78–84	90; 58–110	83; 79–87
2-h glucose (mg/dL)	87; 78–94	94; 89–101	90; 76–108
Basal insulin (μU/mL)	6.2; 4.9–8.2	10; 5.3–11.2	6.7; 5.0–8.9
2-h insulin (μU/mL)	15; 9.9–34	44; 31.1–48	9.9; 7.1–18
HOMA index	1.3; 0.96–1.8	2.1; 1.5–2.3	1.5; 1.1–2.2
Glycated haemoglobin (%)	5.0; 4.8–5.3	5.3; 1.5–9.1	4.9; 4.6–5.2
Subgroup 3 (age >20)			
n	50	8	25
Total cholesterol (mg/dL)	181 ± 30[g]	211 ± 49[g]	126 ± 18
LDL (mg/dL)	107 ± 28[g]	147 ± 46[a,g]	45 ± 13
HDL (mg/dL)	52 ± 17[b]	46 ± 6.5[b]	64 ± 17
Tryglicerides (mg/dL)	86; 76–122	78; 45–132	76; 67–92
Basal glucose (mg/dL)	86; 84–91	91; 81–97	95; 88–113
2-h glucose (mg/dL)	93; 87–100	90; 74–97	96; 83–114
Basal insulin (μU/mL)	4.2; 3.5–14	9.4; 3.9–13	5.6; 5.7–11
2-h insulin (μU/mL)	10; 5.1–35	7.0; 0.5–12	6.9; 6.5–11
HOMA index	0.9; 0.53–3.1	2.2; 0.91–2.7	1.3; 1.2–2.4
Glycated haemoglobin (%)	5.2; 4.9–5.6	5.0; 4.7–5.3	5.2; 4.9–5.6

[a]$P < 0.05$ vs KS; [b]$P < 0.05$ vs controls; [c]$P < 0.01$ vs controls; [d]$P < 0.005$ vs controls; [e]$P < 0.005$ vs KS; [f]$P < 0.0005$ vs controls; [g]$P < 0.0001$ vs controls.
Controls, healthy subjects; HGA, high-grade aneuplodies' patients; KS, Klinefelter syndrome's patients.

The testicles are the glands most affected by these conditions and our hormone data confirmed, in both classic KS patients and in those with HGAs, the presence of hypergonadotropic hypogonadism, caused by primary testicular damage, as demonstrated by the severe hypotrophy and the very low INHB blood levels. In KS patients aged ≤12 years, gonadotropin values were comparable to those of control subjects, while HGA patients showed an initial increase in FSH, with a mean value of 1.5 IU/mL; this was significantly higher than that in KS patients, seeming to indicate an earlier testicular damage.

The expected increase in gonadotropins during late pre-puberty and adolescence (12–20-year subgroup) was seen in both patient populations, with significantly higher values than in the controls (FSH concentrations greater than LH on average). In the same age group, HGA patients had significantly greater values of gonadotropins, while in patients over 20 years, these subjects still had higher levels of FSH and LH than KS patients, but the difference was no longer statistically significant.

There was no difference in total testosterone between KS patients, HGA patients and controls in subjects aged ≤12 years. In contrast, in the 12- to 20-year subgroup, HGA patients showed *T* levels below the normal reference range, and significantly lower than in KS patients and controls. In the oldest subgroup (>20 years), the difference in total *T* (generally in the lower part of the normal reference range) between KS and HGA patients was not statistically significant, but in both groups, it was significantly lower than observed in the control subjects.

As expected, INHB values were similar in all subjects in the youngest subgroup. During late pre-puberty and the first phase of testicular development, there was a dramatic drop in INHB, which was more rapid and severe in the HGAs. In the 12–20 subgroup, KS patients showed significantly higher levels of INHB than HGA patients, in whom the levels were frankly pathological (mean 18 pg/mL vs 5.3 pg/mL), but this difference had disappeared in the oldest subgroup, in which INHB in both KS and HGA patients was significantly lower than in controls.

Evaluation of adrenal gland function by DHEA-S gave contrasting results. In the youngest subgroup, HGA patients had significantly higher DHEA-S values (similar to controls) than KS children, while the opposite was seen in the 12–20 subgroup. In the oldest subgroup, similar DHEA-S concentrations were seen in both KS and HGA patients; these were in the lower part of the normal

reference range, but significantly lower than those in control subjects.

SHBG levels dropped over time in all subjects. In the 12- to 20-year subgroup, HGA patients had significantly higher levels (albeit within the normal range) than KS patients and controls. In the oldest subgroup, both KS and HGA patients showed similar concentrations that were higher than in the controls, but this was only statistically significant in the KS group, probably due to the smaller number of HGA subjects.

The evolution of gonadotropin, T and INHB levels indicated early testicular damage in HGA patients. However, levels of these hormones in KS and HGA patients become similar in adulthood. We hypothesize that the influence of the greater genetic weight of the supernumerary X and Y chromosomes could be responsible for this early testicular failure, but further studies are necessary to better understand the real underlying genetic mechanism.

In our daily clinical practice, a drop in DHEA-S is one of the first signs of late-onset hypogonadism, and is often a precursor to declining T levels (22). DHEA-S as well as T and INHB, showed a premature decline in HGA in comparison with KS patients but, in contrast with our clinical observations of normal karyotype male subjects, its level began dropping concomitantly with testosterone, not earlier.

PRL levels in the youngest age group were normal. In the 12- to 20-year subgroup, PRL was significantly higher in HGA patients than in KS patients and controls, who had similar values. This rise was even more evident in the oldest subgroup, with double the concentration of the 12- to 20-year subgroup. This hormone was pathologically high in 23% and 24% of KS patients in subgroups 2 and 3 and in 62% and 64% of HGA patients in subgroup 2 and 3. This might be explained by hyperactivation of the stress axis, related to the various physical and cognitive difficulties, which affect these patients.

Study of the hypothalamic-pituitary-thyroid axis demonstrated normal secretion of TSH, FT3 and FT4 in KS and HGA patients in all age subgroups. However, higher FT4 in the youngest subgroup and higher FT3 in the 12–20 subgroup were seen in HGA than KS, whereas in the oldest group, the controls had significantly higher FT4 levels, as also demonstrated by Bjørn and coll. (23).

It should also be noted that in the 12–20 subgroup, KS patients had lower FT3 levels than the controls; this difference was not statistically significant (Table 1), probably due to the low number of KS patients. Nevertheless, it confirmed the trend seen in a previous study conducted by our group (24) involving a greater number (40 vs 26) of KS patients in Tanner stage G2-G5. In that case, the higher caseload enabled us to reach statistical significance.

In the present study, Tg values in HGA patients in the youngest age group were significantly higher than those in both KS patients and controls. In the same subgroup of HGA patients, we also found increased FT4 levels. We hypothesize that thyroid function, like prolactin concentration, is strongly influenced by the stress induced by this condition, which is characterized by more severe relational and clinical problems. Furthermore, some studies (25) have established an association between serum Tg values and iodine intake in the iodine-deficient population and suggested that Tg could serve as a sensitive biomarker of iodine deficiency; thus, inadequate iodine intake might also explain the higher Tg levels in our population of younger HGA subjects. Finally, there could be an association between higher Tg levels and decreased thyroid parenchymal integrity. In this study, as well as in our group's previously cited paper (24), we did not find any differences in thyroid autoantibodies between KS and control subjects or between HGA and control subjects (data not shown).

We also studied the lipid profile in terms of total, LDL and HDL cholesterol and triglycerides. Total cholesterol was above the 75th percentile of values for controls in 73% of KS and 55% of HGA patients; HDL was below the 25th percentile of values for controls in 47% of KS and 78% of HGA patients and LDL cholesterol was above the 75th percentile of values for controls in 55% of KS and all HGA patients. Finally, triglyceride concentration was above the 75th percentile of values for controls in 36% of KS and 33% of HGA patients.

Total cholesterol was pathologically high in 3% of KS and 10% of HGA patients, and LDL was pathologically high in 4% and 17% respectively. In contrast, HDL was above the normal reference range in 56% of Klinefelter patients and 67% of HGA patients. Pathological triglyceride concentrations were found in just 3% of KS and no HGA patients.

The statistical analysis revealed significantly higher levels of total and LDL cholesterol in HGA than KS patients, increasing over time. Both had greater concentrations than controls. In contrast, HDL cholesterol was on average higher in KS than HGA patients, although this difference was not statistically significant. No differences in triglyceride levels were seen. Our data are in agreement with much of the literature evidence, that describes increased total and LDL cholesterol and triglycerides and lower levels of HDL cholesterol in KS patients (26, 27, 28).

A number of studies have identified an association between KS and diabetes (29). Despite this, the statistical analysis of our data revealed a slightly increased mean HOMA index in HGA patients in comparison with KS patients, but these values were within normal limits (score <2.5, insulin-resistance cut-off value) (30). Furthermore, impaired fasting glucose was found in just 3 KS patients and no HGA patients.

In conclusion, the hormonal and metabolic data presented in this paper definitively highlight the significant differences between classic KS and HGAs in males. In addition, and supporting our observations, the literature stresses the major clinical, neurologic and cognitive diversities that account for the greater social and clinical care needed by HGA patients (15, 16, 17, 18, 19).

For all these reasons, KS and HGAs of the sexual chromosomes (chromosomes 48 and 49) should be considered as two distinct medical conditions.

Declaration of interest

The authors declare there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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